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THE
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VOL. XXXIII, No. 4 12 OCTOBER, 1934

# STUDIES ON THE RESPIRATION OF CONJUGATING *SPIROGYRA* WITH SPECIAL REFERENCE TO FAT METABOLISM

BY N. L. PAL, M.Sc.
University of Allahabad

(With 17 figures in the text)

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## INTRODUCTION

WHILE working on the respiration of *Spirogyra* in one particular experiment rather low respiratory quotient values were obtained, though normally the ratio found was unity. A careful scrutiny of the experimental procedure revealed nothing that could possibly explain the matter.

An examination of the filaments used in this experiment showed that together with normal filaments there were some conjugating filaments mixed in the sample. The peculiar R.Q. was therefore attributed to the presence of conjugating filaments and the following experiments on the gaseous interchange in *Spirogyra* were performed.

The results are discussed in two sections. Section 1 deals with the respiration of *Spirogyra* in different stages of development and allied problems, while Section 2 deals with the effect of light on the interchange of gases in the same organism.

A search through the available literature showed no record of respiration in conjugating *Spirogyra*.

## MATERIALS AND METHOD

### A. *Collection and treatment of the material*

All the experiments were performed with *Spirogyra*, except two that were performed on *Sirogonium*. The organisms were collected locally from two adjacent ponds.

The *Spirogyra* collected for the experiments consisted probably of more than two species. But in most cases they were collected from a single spot, and in a single collection one species generally predominated. Sometimes a few other organisms were found mixed with the *Spirogyra*, but cases where they were abundant were rare. Collections thus contaminated with other filaments were always discarded.

The collected filaments were brought to the laboratory, placed in a flat dish of filtered pond water and kept in the light near a glass window for 2 days, previous to an experiment. Before an experiment was started, the filaments were examined under a microscope to note their condition. If they were not healthy, or if the filaments were contaminated with other organisms, the experiment was not started but a fresh collection was made.

The filaments were carefully washed in distilled water, changing the liquid several times. They were then placed in the apparatus for

20 min. without taking any readings, to allow the plants to get adapted to the new environment. Then the experiment was started in the same liquid which was previously boiled and cooled in an atmosphere devoid of carbon dioxide.

### B. *The apparatus*

Winkler's method of estimating oxygen has been widely used for experimenting on the gaseous interchanges of water organisms. A difficulty arises, however, from the fact that the amount of oxygen dissolved in a sample of water is greatly affected by even very slight contacts with the atmosphere.

A very simple apparatus specially suited for biological experimentations was described by Osterhout and Haas(15) in 1917. Although the difficulty of estimating oxygen is removed the apparatus is not an ideal one. In the first place, it does not allow the organisms a supply of continuously flowing liquid, which is essential for an experiment continued for a long time. In the second place, their method of introducing T-tubes for consecutive readings not only makes the apparatus difficult to handle but also renders the calculations complicated. Moreover, introduction of additional T-tubes becomes necessary for estimations of carbon dioxide interchange.

In view of the above difficulties, the apparatus described below was devised which gave very accurate results.

The apparatus (see Fig. 1) consists of the following parts: the thermostat bath; the reservoir and plant chamber; and two sets of collection tubes.

The thermostat water bath (*A*) is provided with a burner and a mercury flame regulator to keep the reservoir (*B*) and the plant chamber (*C*), which are immersed in it, at a constant temperature.

The reservoir (*B*) is a glass vessel, the capacity of which depends upon the kind of experiment and its duration. For algal work a 5-litre flask or bottle is very convenient. The plant chamber is a small cylindrical vessel (*C*) about 6 cm. diameter and 3 cm. high. There are two tubes attached to the plant chamber, one coming from the reservoir and the other, which is a capillary one, reaching the collection tubes *D* as shown in the figure. Between the plant chamber and the tube *D* is inserted a small capillary tube *T* about 6 cm. long provided with two screw clamps (*a* and *b*), one at each end.

Each set of collection tubes consists of three parts, *D*, *E* and *F*, mounted on a stand, *D* and *E* being removable. *D* is the oxygen estimating tube about 9 cm. long and having a capacity of 50 c.c. It

has three tubes fused with it. The first is a capillary one at the top connecting the plant chamber, the second connecting the tube *E* beneath, and the third about 1·5 cm. diameter and 2 cm. long at one side. This side connection which serves as an opening for the introduction of chemicals is provided with a screw clamp (*c*). The connection between *D* and *E* is also provided with a screw clamp (*d*).

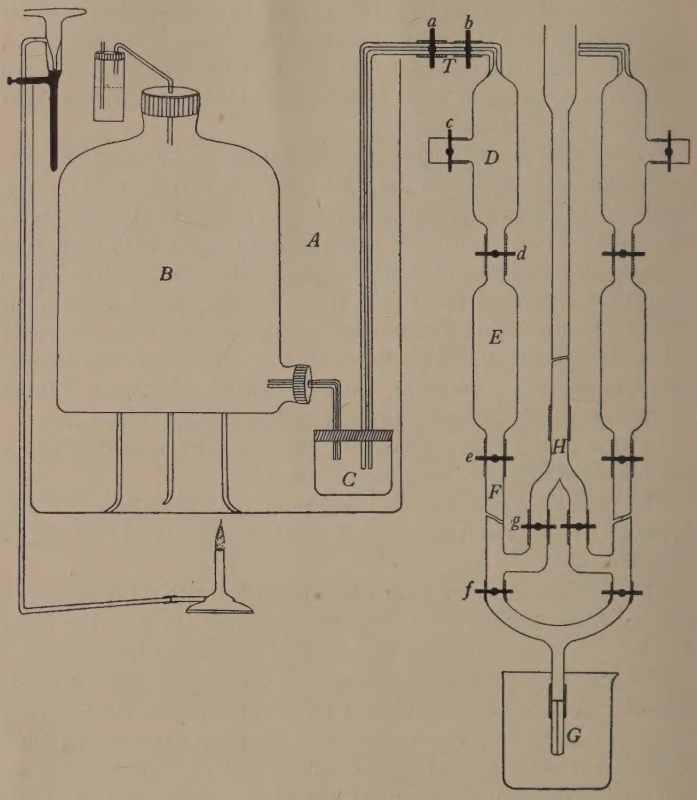



Fig. 1.

*E* is a tube similar to *D* and of the same size but without the side tube attached to it. *E* communicates with *F* beneath through the clamp (*e*).

*F* is a long tube about 30 cm. long and 1 cm. diameter with a side tube bent at right angles attached to it towards the lower end. The lower ends of *F* and the corresponding tube of the other set are

joined to two arms of a Y-tube through clamps (*f*). The third arm of the Y-tube is attached to a capillary tube *G*. This capillary tube is so graduated that if the tubes *D*, *E* and *F* are all filled with mercury and the clamps opened, about 100 c.c. of mercury will fall out in drops every hour. A tube of about 1 mm. bore, partially fused at one end to close the aperture to the desired diameter, is better suited for the purpose than a capillary tube of very fine bore. The tube thus fused is inserted in the apparatus with the open end outside serving as the outlet for mercury. This arrangement prevents the clogging of the bore and also spilling of mercury as it falls in drops.

The side tubes attached to *F* and its corresponding tube of the other set, referred to above, communicates through another Y-tube with *H*, which is a long tube serving as the filler and reservoir of mercury. These connections are also provided with clamps (*g*).

*Manipulation of the apparatus.*

The bath is first adjusted to the desired temperature. The reservoir bottle *B* is filled with the solution to be provided to the plants and the plants are placed in the chamber *C*. The stopper of the plant chamber is now tightly closed, taking care that the chamber is free of air bubbles. Both the reservoir and the plant chamber are now placed in position in the thermostat bath. In order that the reservoir may not communicate with the atmosphere directly, it is connected to a small bottle of water as shown in Fig. 1. Water is sucked out from *T* and allowed to flow out for some time to enable the plants to become adjusted to the new conditions, and then the clamp (*a*) is closed.

In the meantime the collection tubes are filled with mercury. This is done in the following manner. Mercury is poured in the tube *H* from above, the clamp (*g*) and its corresponding one being closed previously. By opening *g* carefully mercury is allowed to fill up the tubes *F* and the Y-tube beneath it. Clamp *f* and its corresponding one are now closed.

Mercury is now allowed to fill the tubes completely by opening *g* fully, taking care to close the side connection (*c*) previously. To avoid spilling the mercury, a tube bent upwards is inserted at *b*. When mercury reaches this tube, *b* is closed and the tube is carefully taken out.

*T* is now set in position. Clamps *a*, *b* and *f* are opened and mercury begins to drop from *G*, thereby drawing in water in the tubes *D* and *E* from the plant chamber.

When both *D* and *E* are thus filled with algal water and the

mercury level stands at a marked position in *F*, clamps *f*, *b* and *a* are closed. *T* is now taken out from the set and inserted in the other set which is kept ready previously filled with mercury. The necessary clamps are now opened so that mercury again begins to drop and algal water collects in the tubes of the other set.

From the volume of *D*, *E* and *F* up to the marked position and the time required to fill them with algal water the rate of flow is previously adjusted to about 100 c.c. per hour. As the total volume of *D*, *E* and *F* is also 100 c.c. one set of collection tubes works for about an hour, which time is utilised in estimation of oxygen and carbon dioxide in water collected in the previous set.

*Estimation of oxygen and carbon dioxide.*

Oxygen is estimated by Winkler's method as adapted by Osterhout and Haas. Clamp *d* is closed and the tube *D* is taken out from the stand. Reagents are now introduced in a manner identical to that of Osterhout and Haas.

A small glass tube about 3 cm. long and 1 cm. diameter is inserted at the side tube. Alkaline solution of potassium iodide is introduced in this tube until the level reaches up to the rubber tube attached to it. The free end is now closed by means of a clamp, taking care that no air bubble is introduced. The excess of liquid is poured off and the rubber is well washed first in running water and finally with distilled water. The clamp between *D* and the tube introduced is now opened.

A second tube is now introduced at the free end of the first and solution of manganese chloride is similarly introduced and the outer clamp closed. The clamp between the first and the second tube is opened and the apparatus inverted several times so that the reagents are well mixed. After washing the free end carefully as in the previous case the apparatus is left for about 15 min. to complete the reactions.

Then by means of a third tube about 2 c.c. sulphuric acid is introduced into the apparatus. When the precipitate previously formed is dissolved the whole solution is poured off in a beaker and the amount of iodine liberated is titrated against standard sodium thiosulphate solution, using starch as indicator. From this the amount of oxygen dissolved in the sample of water is calculated.

The strengths of the reagents required depend upon the amount of oxygen dissolved. The following has beenf ound to be quite suitable for experiments with algae:

(1) 10 grm. of KI in 100 c.c. 36 per cent. NaOH.
(2) 33 per cent. $MnCl_2$.
(3) $H_2SO_4$ diluted 1 : 1.
(4) $N/100$ $Na_2S_2O_3$.

Carbon dioxide is estimated by addition of excess of barium hydroxide solution, and then titrating the excess amount by standard HCl.

By means of a pipette, 20 c.c. of algal water is taken in a flask. The latter is provided with a stopper having two bores. Through the first bore is introduced a glass tube, one end of which is kept dipping in water in a beaker. Through the second, the end of a micro-burette (graduated to measure 0·05 c.c.) is introduced. A definite amount of $Ba(OH)_2$, to which phenolphthalein has previously been added, is introduced into the flask from the burette. The flask is taken out and immediately connected to another micro-burette containing standard HCl. The excess of $Ba(OH)_2$ is now titrated. The titrated liquid is kept in a stoppered flask and used as a standard for comparing the colour while taking the end-points in subsequent titrations.

The solutions used for algal experiments are

(1) $N/150$ $Ba(OH)_2$.
(2) $N/200$ HCl (standard).

### C. *Source of light*

In the experiments with light an Osram 1000-watt electric bulb was used. The bulb was placed at a distance of 15 cm. from the plant chamber. Between the plant chamber and the bulb was placed a rectangular jar about 10 cm. wide filled with water to absorb the heat rays. The water in the jar was kept circulating by a connection from a tap and a siphon arrangement to the sink. The bulb was surrounded by a reflector.

The thermostat bath in which the reservoir and the plant chamber were kept immersed had a small glass window for the entry of the light. The chamber was placed with the flat surface at right angles to the light rays.

For the experiments in the dark the plant chamber was kept covered with a thick piece of black cloth.

The results obtained in all the experiments were referred to the dry weights of the plants. When an experiment was over the algae were taken out in a weighing tube and placed in an electric oven maintaining a temperature of 95–96° C. The weighings were made

after a period of 24 hours and were then constant. To this dry weight the activity of filaments was referred. In all the experiments the temperature of the bath was maintained at 35° C.

## SECTION 1. RESPIRATION OF *SPIROGYRA* IN DIFFERENT STAGES OF CONJUGATION

### A. *Experimental results*

(*a*) *Respiration of conjugating* Spirogyra.

In all, six experiments were performed with *Spirogyra* and two with *Sirogonium*. These will be taken up one by one.

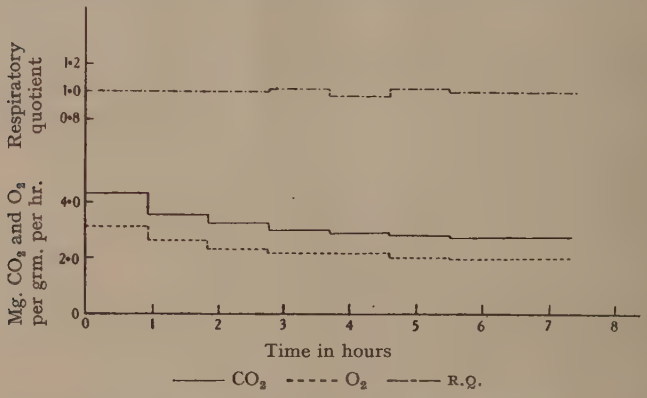



Fig. 2. Respiration of *Spirogyra* without conjugation.

Fig. 2 represents the respiration of normal *Spirogyra* filaments. The respiration intensity at the beginning of the experiment is 4·31 mg. carbon dioxide and 3·13 mg. oxygen per grm. (dry wt.) per hour. It gradually falls off to the lower values of 2·74 mg. carbon dioxide and 1·99 mg. oxygen at the end of the experiment. The respiratory quotient throughout the experiment remained near unity—the very slight departures being entirely due to experimental errors.

The algae employed in this experiment were healthy, fully green and slimy to touch, and remained so till the experiment was over. In this collection there was no cell conjugating or sending out conjugation tubes.

Fig. 3 represents the respiration curve of a mixture of vegetative and conjugating filaments. The conjugating filaments were roughly about 50 per cent. of the total quantity taken and were in a very

early stage of conjugation. The output of carbon dioxide was 2·72 mg. per hour, while the oxygen intake was as high as 4·90 mg. per hour. Because of the high oxygen intake the respiratory quotient came down to the low value of 0·5. The respiratory intensity, however, remained fairly constant throughout the experiment.

The rest of the experiments were performed with conjugating filaments alone. This was quite easily managed, as the filaments of

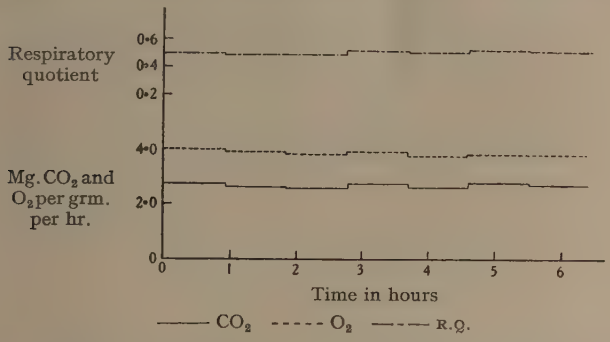



Fig. 3. Respiration of *Spirogyra*. 50 per cent. of the filaments conjugating.

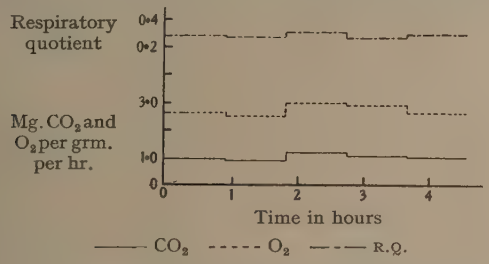



Fig. 4. Respiration of conjugating *Spirogyra*. Conjugation tubes formed but migration of the protoplasm not yet begun.

*Spirogyra* generally undergo conjugation simultaneously, leaving out very few vegetative filaments. The few normal filaments that were present originally in the sample were, however, carefully removed under a dissecting microscope before the experiments were started. It is to be noted here, however, that though generally a great number of cells of a filament take part in conjugation, there are almost always some inactive cells which fail to produce a conjugation tube, thus leaving a few vegetative cells in the filament.

Fig. 4 represents the respiration of filaments, the cells of which have already sent a good number of conjugation tubes. Very few

cases of passage of protoplasm from one gamete to the other had taken place in this collection. The chlorophyll bands in most cases were intact though a bit disorganised. The intensity of respiration was much lower, being only 0·97 mg. carbon dioxide and 2·57 mg. oxygen, which kept nearly level throughout. The respiratory quotient was as low as 0·27.

Instead of destroying the filaments used in the last experiment, they were placed in pond water in a well-lighted place at room temperature for 2 days. After the expiry of the period, an examination under the microscope revealed the fact that the process of conjugation had in the meantime far advanced and a great number of protoplasmic migrations and some zygospore formations had taken place. These filaments were now put in the respiration chamber and the experiment started.

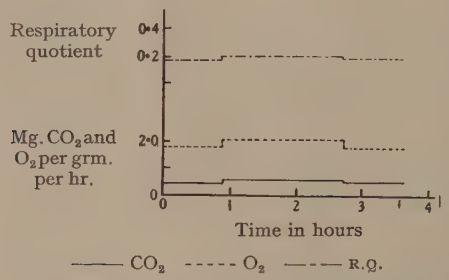



Fig. 5. Respiration of conjugating *Spirogyra*. Protoplasmic migration has begun.

In this experiment (Fig. 5) both the carbon dioxide output and the oxygen intake fell off to about 0·5 mg. carbon dioxide and 1·8 mg. oxygen. The R.Q. also fell to 0·2.

In the next experiment filaments at a very advanced stage of conjugation were employed. The content of all gametes had rounded off, and in the majority of cases the protoplasm of one had travelled to the other. Numbers of fully formed zygospores were also present. The results are shown in Fig. 6. The carbon dioxide output and oxygen intake and the R.Q. all remained low as in the previous experiment.

Two experiments were now performed with *Sirogonium*, another member of the Conjugatae. The filaments were collected from the same pond and were treated in a similar manner.

Fig. 7 represents the respiration of *Sirogonium* filaments without any conjugation. The intensity is 3·21 mg. carbon dioxide and 2·33 mg.

oxygen. These do not show a falling tendency in agreement with the normal *Spirogyra* filaments. The R.Q. is near unity as in *Spirogyra*.

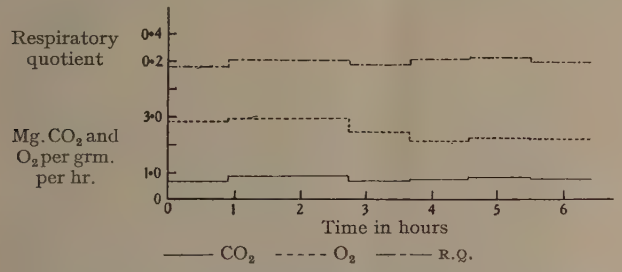



Fig. 6. Respiration of conjugating *Spirogyra*. Many cases of zygospore formation seen.

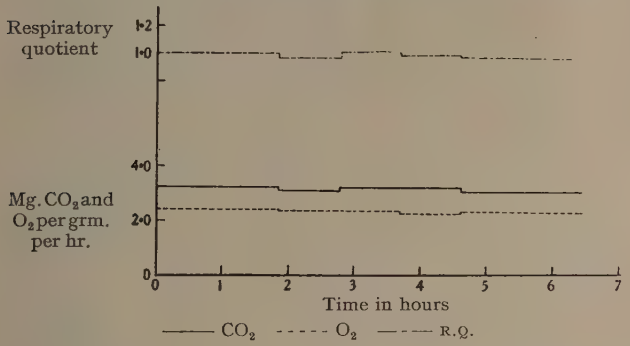



Fig. 7. Respiration of *Sirogonium* without conjugation.

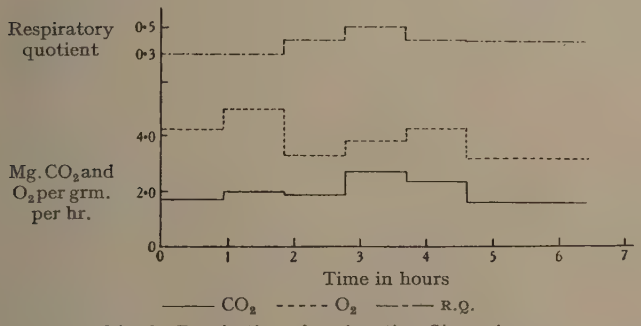



Fig. 8. Respiration of conjugating *Sirogonium*.

Fig. 8 gives the respiration of conjugating *Sirogonium*, in which it should be noted that only a few cells take part in conjugation. The

respiratory intensity is lower than in the previous case, being about 2·0 mg. carbon dioxide and 3·5 mg. oxygen. The R.Q. falls to the neighbourhood of 0·38.

(*b*) *Oil and starch content in conjugating* Spirogyra.

A search through the available literature showed no record bearing on the physiology of conjugation. Low R.Q. values have, however, been obtained in many plants, especially when fats and oils are utilised for respiration purposes. Many investigators have found a great accumulation of oil in the zygospores of members of the Conjugatae (22). Though it is stated that during germination the oil present in the zygote is reconverted to sugar, the exact time of formation of the oil found in the zygote has not yet been worked out. Fritsch (22), however, reported the presence of oil in the gametes of *Sirogonium* before actual fusion of the gametes.

Therefore, in order to know how far oil was responsible for the peculiar R.Q. obtained, a few chemical and microchemical examinations were made.

For tests of oil (24), osmic acid (1 per cent.) solution and Sudan III (saturated solution in 70 per cent. alcohol) were used. Starch also was simultaneously tested for by chloralhydrate iodine solution.

It was found that the vegetative *Spirogyra* contained abundant starch in the chloroplasts but was devoid of any oily matter. (Osmic acid, however, stained the cells black owing to the presence of a great amount of tannin, the presence of which was also proved by staining with ferric chloride (24). However, when tannins were removed by boiling with water and oil was tested for by osmic acid no colour was developed.)

Filaments undergoing conjugation, examined after staining with iodine, showed the presence of starch in all filaments. But starch was more abundant and much larger blue patches were formed in the cells that had sent out conjugation tubes. Cells not so far advanced and those that failed to form conjugation tubes had smaller and fewer starch grains. Stained with Sudan III, the general cytoplasm of the vegetative cells adjoining the conjugating ones turned slightly pinkish, showing diffused oily matter present in the cells. On the other hand, the cells which had conjugating tubes when stained with Sudan III showed big oil globules arranged in the chloroplasts mixed with hyaline globules which were proved to be starch grains by staining with iodine (Fig. 9). Osmic acid failed to colour these oil globules immediately, but on keeping a deep black colour was obtained.

Estimations of ether-soluble matter in *Spirogyra* confirmed the formation of oil during the maturation stage. The algae were first dried at 95–96° C. for 24 hours. The dried material was well crushed in a mortar and ether was added to it. The solution was filtered off and new ether added and the same operations repeated until no soluble matter was left in the material. The method is rather crude, and does not give the absolute amount of oil in a sample, but serves well to show any change in fat content, provided the amounts of other ether-soluble substances remain unchanged.

The ether-soluble matter thus obtained in normal *Spirogyra* was 3·4 per cent. of the dry weight; and in two samples of conjugating

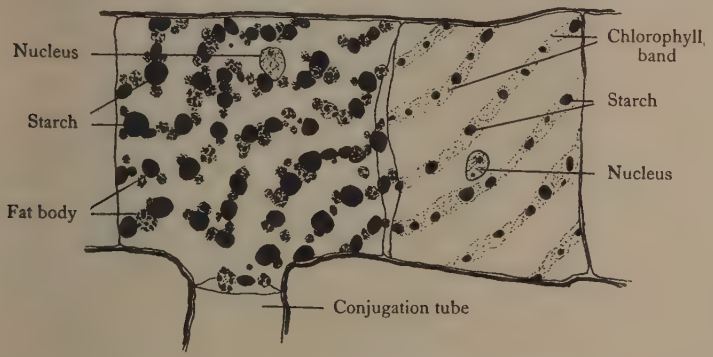



Fig. 9. Part of a conjugating filament of *Spirogyra*.

filaments in which there were no zygospores was 6·1 per cent. in one case, and 5·2 per cent. in the other.

Estimations of reducing sugars were also made both in normal and conjugating filaments by Pavy's solution, but in both cases the amounts were so small that no definite results were obtained.

(*c*) *Diastatic and lipasatic activity in* Spirogyra.

Both normal and conjugating filaments were now examined for diastatic and lipasatic activity. The filaments were hand pressed to squeeze out extra water and then weighed. Thrice the weight of water was now added and the solution was allowed to stand for a few minutes. Then it was filtered and tested for the enzymes in the following manner.

Diastatic activity was estimated by Wohlgemuth's iodine method (20). Various concentrations of enzyme solutions were taken in uniform test-tubes and 5 c.c. of enzyme solution and 0·5 c.c. toluol

were added to each. The tubes were then well shaken, stoppered and incubated at 40° C. for 20 hours. After the period of incubation, they were immediately cooled and three drops of $N/10$ iodine solution added. The tube of highest dilution which developed a blue-red colour was taken to represent the limit of enzyme activity. The results are given in Table I, which were identical in both normal and conjugating filaments.

TABLE I

| Enzyme c.c. | 5 | 2 | 1 | 0·5 | 0·3 | 0·2 | 0·1 |
|---|---|---|---|---|---|---|---|
| Iodine reaction | Yellow | Yellow | Yellow | Red-yellow | Blue | Blue | Blue |

Thus the diastatic activity in both normal and conjugating filaments was $\left(\begin{matrix}40° \text{ C.} \\ D \ 20 \text{ hours}\end{matrix}\right)$ 10.

The lipasatic activity was estimated by Kanitz' method (20). A little phenolphthalein was added to some commercial olive oil which was shaken with a few drops of decinormal sodium hydroxide until a uniform emulsion of slightly pinkish colour was obtained. 10 c.c. of this emulsion were taken in different flasks containing 5 c.c. of enzyme solutions. These were now incubated at 5° C. for 24 hours. Similar flasks were taken in which enzyme solution was added after boiling for about 2 min. After the period of incubation the flasks were taken out and 50 c.c. of 95 per cent. alcohol and 5 c.c. ether were added to each. The mixtures were then titrated against standard decinormal sodium hydroxide. The average of the controls were subtracted from the average of the flasks containing unboiled enzyme and the amount of acid produced calculated.

It was found that the lipase activity of normal *Spirogyra* filaments without any conjugating ones was negligible. But, in conjugating filaments, 5 c.c. of the enzyme solution converted 92·9 mg. oleic acid in 24 hours.

Thus, in the conjugating filaments, the diastatic activity remains equal to that of normal filaments but the lipasatic activity which is absent in normal filaments greatly increases in the conjugation stages.

### B. *Discussion*

The *Spirogyra* filaments require a few days' time to pass through all stages of conjugation, and as will be shown in Section 2 of this paper darkness greatly hinders this process. Hence it is not possible

to estimate the gradual drift of respiratory intensity in conjugating *Spirogyra* in a single experiment.

However, by joining the initial respiratory values obtained in different experiments with *Spirogyra* in various stages of conjugation one can construct a hypothetical curve showing the respiratory intensities at these stages. Fig. 10 has been constructed in the manner mentioned above, showing the initial carbon dioxide output, oxygen intake and the respiratory quotient at these different stages. A fairly good idea of the respiratory mechanism can be obtained from these curves.

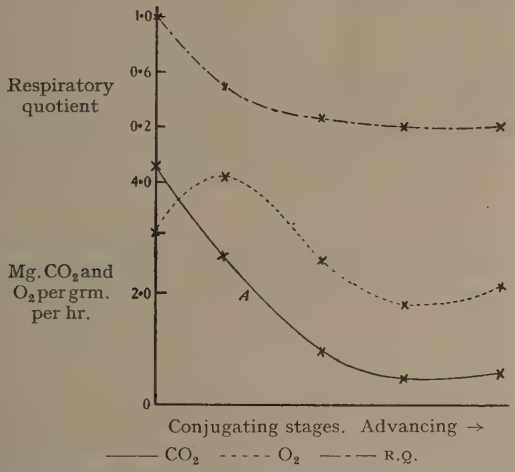



Fig. 10. Respiration of conjugating *Spirogyra* at different stages.

It is now proposed to examine the points of interest arising out of these curves separately.

(*a*) *Intensity of carbon dioxide emission at different stages of conjugation.*

The unbroken line in Fig. 10 represents the carbon dioxide emission per grm. per hour at different stages of conjugation. It will be noticed that in normal *Spirogyra* the respiratory intensity is comparatively high, being about 4·3 mg. $CO_2$ per hour. However, as the filaments send out conjugation tubes, a physiological change takes place in the protoplasm and the carbon dioxide emission falls off, the intensity being about 1 mg. $CO_2$ by the time the conjugation tubes are fully formed. By the next stage, when the cholorophyll bands disorganise and the cell contents begin to pass off, the intensity falls

still lower to the value of 0·5 mg. $CO_2$, and thereafter remains fairly constant. The lowest value reached is only about 12·5 per cent. of the intensity in normal filaments.

At first glance, this very steep fall in the amount of carbon dioxide given off seems to be very confusing. The protoplasm in these stages is in quite an active state. The movement of the filaments for the side by side arrangement, the formation of the beak, the internal changes in the cell contents and finally the passing out of these contents, all require, we should expect, a great amount of energy. But the carbon dioxide emission is only 12·5 per cent. of that of normal filaments. If the energy release is proportional to the carbon dioxide production, only 12·5 per cent. of the energy released in normal *Spirogyra* is available during the final stages of conjugation.

This seems to be an abnormally low figure in view of the protoplasmic activity. Moreover, the experiments have shown that the lipase activity actually increases and the diastatic activity remains constant in these stages, showing that the enzyme activities of the filaments are in no way deficient.

At this stage, therefore, the only conclusion that can be drawn is that the carbon dioxide emission is not a true measure of the total amount of energy released in the conjugating phase. This point will again be taken up later in this paper.

(*b*) *The oxygen intake in conjugating* Spirogyra.

The dotted line in Fig. 10 represents the oxygen intake in *Spirogyra* at different stages of conjugation. In normal filaments it is about 3 mg. per hour. But as the conjugating tubes appear this value first mounts to 4 mg. per hour and then it begins to fall until the zygospore formation stage is reached, when it remains at about 2 mg. per hour. The uneven nature of the curve is fundamentally different from the carbon dioxide output curve which shows a continuous fall from the beginning. It is evident that the relation between the oxygen intake and the carbon dioxide output is not constant as in normal plants.

The higher amount of oxygen intake and the smaller fall in this value may be due to the fact that the oxygen taken in, during the conjugation stages, is used in reactions other than that of oxidising sugars completely into carbon dioxide and water. This will be further clarified under the next heading.

(*c*) *The respiratory quotient in conjugating* Spirogyra.

The dot and dash line in Fig. 10 shows that like the carbon dioxide output the respiratory quotient values also fall off with advancing stages of conjugation. This, together with the lower carbon dioxide emission, strongly suggests that there is a great change in the metabolic activity in conjugating *Spirogyra*.

Respiratory quotient values lower than unity have been obtained in many plants, specially in those which use fats and oils as a substrate for respiration. Some results of this kind obtained by different authors are given below.

| Organisms | R.Q. | Authors |
|---|---|---|
| 1. *Phycomyces* grown on ground linseed medium | 0·65–0·75 | De Boer(3) |
| 2. Lupin seeds | 0·54 | Bonnier and Mangin(1) |
| 3. Flax | 0·3–0·64 | Bonnier and Mangin(1) |
| 4. Linseed | 0·64 | Gerber(5) |
| 5. White mustard seed placed in different concentrations of $CO_2$ | 0·45–0·82 | Kidd(10) |

Very recently, Stiles and Leach(19) have conducted estimations of R.Q. in different seeds with their improved katharometer. They also find that those containing fat reserves have very low R.Q. values.

Such low values are obtained as a consequence of the low oxygen content in fats, which require a great amount of oxygen for complete oxidation. For example, when triglyceride of ricinoleic acid (the chemical nature of the fat found in *Spirogyra* being unknown, this fat has been used for all considerations and calculations in this paper) is oxidised according to the following equation:

$$2C_{57}H_{104}O_9 + 157O_2 = 114CO_2 + 104H_2O,$$

the R.Q. would be only $114/157 = 0{\cdot}73$.

The presence of fat at the conjugation stage in *Spirogyra* and *Sirogonium* and the considerably greater lipasatic activity support the view that the low R.Q. obtained may be due to the oxidation of this material.

But the R.Q. should not, in ordinary cases, be much lower than 0·7 when the fat is completely oxidised to carbon dioxide and water, whatever fat is used for the purpose. Therefore, such extreme values as 0·2 cannot be explained by the simple oxidation of fats to carbon dioxide and water.

Regarding the chemistry of oxidation of fats in plants nothing as yet is definitely known. It is, however, a well-established fact

that, in seeds, the carbohydrates begin to accumulate as the fats disappear. Thus carbohydrates are formed by conversion of fats. This was observed by de Saussure as far back as 1842 and has since then been confirmed by various authors. This partial oxidation of the fats requires a great amount of free oxygen.

Thus the very low R.Q. values may be due to the conversion of fats to carbohydrates. Stiles and Leach(19) have shown that if only half of a given amount of the triglyceride of ricinoleic acid is oxidised to carbon dioxide and water and the other half converted to sugar according to the following theoretical equation:

$$2C_{57}H_{104}O_9+10H_2O+43O_2=19C_6H_{12}O_6,$$

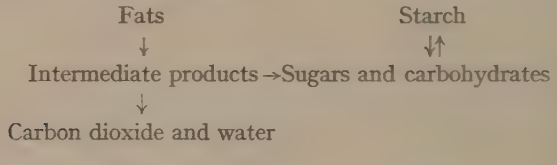
the R.Q. would be only $114/157=0{\cdot}57$. Similarly, if only a third part is respired while the rest is converted to sugar the R.Q. would fall to 0·47.

Thus it may be concluded that the very low R.Q. in conjugating filaments is due to a very rapid conversion of oils into carbohydrates, by which process a great amount of oxygen is fixed without any liberation of carbon dioxide.

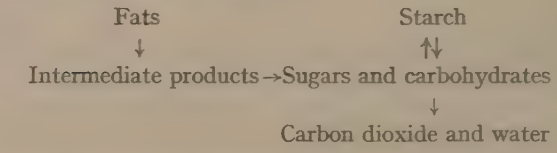
(*d*) *Substrate used for respiration in conjugating* Spirogyra.

That in normal *Spirogyra* the substrate used for respiration is sugar as in all other plants is shown by the fact that the R.Q. in these filaments is unity. However, in the conjugating ones it is found that oils take a great part in the metabolism. It has also been pointed out that the oil is not completely oxidised to carbon dioxide and water, but a great amount of it is converted to sugar. These changes can take place in two different ways:

(1)




(2)




According to the first scheme the fats are directly oxidised to carbon dioxide and water without being converted to sugars. Thus the substrate for respiration becomes fat and not sugar, though, even

in this case, a considerable amount of fat is converted to sugar. The point, therefore, at once arises why the normal respiratory process should stop and instead of carbohydrates which are abundant, the oils should begin to be utilised for the purpose.

As has been pointed out before, in no stage of conjugation are the filaments very deficient in starch content. The diastatic activity also remains constant throughout, showing that starch ⇄ sugar reaction is not greatly altered in conjugating filaments. Therefore in view of the abundance of starch and simple carbohydrates it does not seem probable that the oxidation of sugar to carbon dioxide is greatly affected by light, which is a finding contrary to the first scheme.

According to the second scheme, however, the fats are first oxidised to carbohydrates, which are themselves then further oxidised to carbon dioxide and water. Thus the normal respiratory process continues, and only a side reaction of conversion of fats to carbohydrates is added, which necessitates a high intake of oxygen thereby greatly lowering the R.Q. The greater the magnitude of the side reaction, the lower becomes the R.Q.

Though both the schemes are possible, the second one seems to be more probable in view of the above considerations.

(*e*) *Fate of oxygen taken in.*

From the above it is seen that the oxygen taken in is used for two separate reactions:

(1) For the oxidation of sugars to carbon dioxide and water, and

(2) For conversion of oil to sugar and carbohydrates.

The amount of oxygen used for the first reaction can be calculated from the carbon dioxide emission of the algae on the basis that the ratio between the volumes of the two gases is unity. Then subtracting this amount from the total amount taken in, one can also have a measure of the amount used in the second reaction. Thus the total quantity of oxygen taken in can be broken up into two component parts. Table II gives these calculated values.

TABLE II

| Experiment represented by Fig. no. | Carbon dioxide output mg. | Amount of oxygen taken in mg. | Oxygen used for oxidation of sugar mg. | Oxygen used for conversion of oil mg. |
|---|---|---|---|---|
| 2 | 4·31 | 3·12 | 3·12 | — |
| 3 | 2·72 | 4·09 | 1·98 | 2·11 |
| 4 | 0·97 | 2·57 | 0·71 | 1·86 |
| 5 | 0·44 | 1·77 | 0·32 | 1·45 |
| 6 | 0·30 | 1·41 | 0·41 | 1·00 |

17-2

Fig. 11 gives the amounts of oxygen used for oxidation of sugars (*A*) and for conversion of oil (*B*) separately in a graphical form. It will be seen from this that while *A* necessarily corresponds with the carbon dioxide curve of Fig. 10, *B* is much smoother than the oxygen curve of Fig. 10. The amount of oxygen used for the conversion of oil keeps fairly constant, thereby showing that the reaction goes on practically at constant rate. Thus the uneven nature of the total

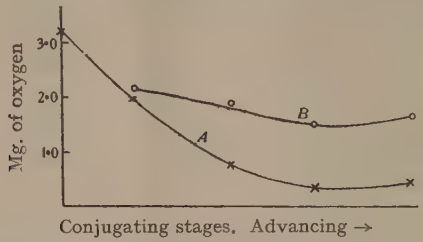



Fig. 11. Fate of oxygen taken in by conjugating *Spirogyra*. *A*. Oxygen used for oxidising sugar. *B*. Oxygen used for conversion of fat to carbohydrates.

quantity of oxygen taken in is only due to the summing up of the amounts used for two separate reactions and not due to any great uneven variation in any of these.

(*f*) *True index of energy released in conjugating* Spirogyra.

Ordinarily, the amount of carbon dioxide emission is taken to be the measure of respiratory intensity. However, respiration in its true sense includes all those reactions which release energy for the use of the organisms. As previously shown oils are oxidised to carbohydrates in conjugating *Spirogyra*. Oils are of higher energy content than the carbohydrates and therefore in this reaction a great amount of energy is liberated. The following considerations are therefore necessary in order to understand the true respiratory intensity in *Spirogyra*.

When a gram of glucose is oxidised to carbon dioxide and water 3·76 K. of heat is given out; therefore, in the following reaction,

$$C_6H_{12}O_6+6O_2=6H_2O+6CO_2$$

per gram of oxygen used $3{\cdot}76\times180/192=3{\cdot}525$ K. of heat will be given out.

Similarly, the energy given out when the fats are oxidised to carbohydrates can be calculated.

Every gram of fat when oxidised to carbon dioxide and water gives out 9·1 K. of heat;

$$2C_{57}H_{104}O_9+157O_2=114CO_2+104H_2O,$$

i.e. 1864 grm. of fat when oxidised will give out 9·1 × 1864 K. of heat.

But in *Spirogyra* the reaction stops at the carbohydrate stage; thus part of the energy that would have been free is utilised in the formation of sugars,

$$2C_{57}H_{104}O_9 + 10H_2O + 43O_2 = 19C_6H_{12}O_6.$$

Thus 1864 grm. of fat gives 19 × 180 grm. of sugar, which has an energy content of 19 × 180 × 3·76 K. of heat. Therefore in the above reaction 1864 grm. of fat and 180 grm. of water combining with 1376 grm. of oxygen gives out 9·1 × 1864 K. + 587 K. (energy content of water)—19 × 180 × 3·76 K. = 4690·2 K. of heat. Thus 1 grm. of oxygen utilised in the above reaction gives 4690/1376 = 3·4 K. of heat.

Thus from the amount of oxygen used in the different reactions of Table II the actual amount of energy released can be calculated. Table III gives these values.

TABLE III

| Experiment represented by Fig. no. | Oxygen used for oxidation of sugars mg. | Oxygen used for conversion of fat (mg.) | Energy from oxidation of sugars | Energy from fats | Total amount of energy |
|---|---|---|---|---|---|
| 2 | 3·12 | — | 10·98 K. | — | 10·98 K. |
| 3 | 1·98 | 2·11 | 6·97 K. | 7·17 K. | 14·14 K. |
| 4 | 0·71 | 1·86 | 2·50 K. | 6·32 K. | 8·82 K. |
| 5 | 0·32 | 1·45 | 1·13 K. | 4·93 K. | 6·06 K. |
| 6 | 0·44 | 1·66 | 1·55 K. | 5·64 K. | 7·19 K. |

The total energy released in different stages is represented in Fig. 12. The curve at first shows a rise, then a fall, finally ending in a level

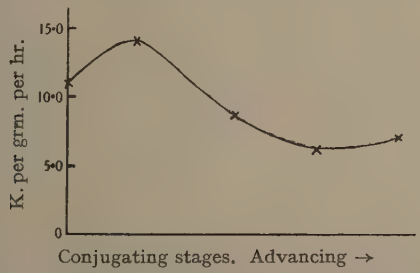



Fig. 12. Energy released in conjugating *Spirogyra*.

course. Therefore the conclusion arrived at is that the activity of conjugating *Spirogyra* increases in the first stage while the beak is

being formed. This is followed in the second by the gradual slowing down of the activity while the cell contents begin to disorganise. While in the third stage, when the protoplasm of one of the gametes begins to pass off to the other, the activity is about the lowest, being roughly half the intensity of that of normal filaments. This finding is quite in accordance with what is expected; for in the first stage, owing to the movement of the filaments for side-by-side arrangement and the formation of the beak, the protoplasm is in an intensely active state; while in the third stage, the protoplasm gradually stops all normal functions and is in a low state of activity.

A comparison of the energy release curve and the carbon dioxide curve would bring out the great divergence between the two. Thus it is evident that, in conjugating *Spirogyra*, the carbon dioxide emission curve cannot be employed as an index of the metabolic activity. While, on the other hand, the oxygen intake is to some extent proportional to the energy release and therefore its study can to some extent serve the purpose, though for a full investigation an analysis of the actual amount of heat developed would be necessary.

(*g*) *Oil reserve in* Spirogyra.

The foregoing discussions therefore lead us to conclude that during the process of conjugation, part of the oil present is oxidised to sugar. But as has been pointed out before, the staining tests and the estimations of the ether-soluble matter have shown that actually fats begin to accumulate only when conjugation tubes are being protruded.

At this stage, the only explanation possible is that oil begins to form just before conjugation starts and its presence is overlooked at the time. This previously accumulated oily matter is oxidised again in the conjugation stages. But such reactions are highly improbable in view of the fact that a great amount of oil is found in the zygote. Moreover, in that case, there should be a certain stage when owing to the formation of oil the R.Q. would be greater than unity. Though several experiments were conducted to find the stage out, in no experiment was the R.Q. obtained greater than unity; it was either unity (in normal filaments) or less than unity (in filaments in any stage of conjugation).

It was later found that the reversibility of the reactions was greatly influenced by light; and the darkness inevitable for respiration experiments was responsible for the conversion of oil to sugar. This point will be taken up further when dealing with the light experiments.

## Section 2. Effect of light on the physiology of conjugation

### A. *Experimental results*

The following four experiments were now performed with light. The method of experimentation has been dealt with in the introductory part of this paper.

In the first three experiments normal *Spirogyra* filaments without any lateral attachments were employed, while in the last experiment conjugating filaments were taken.

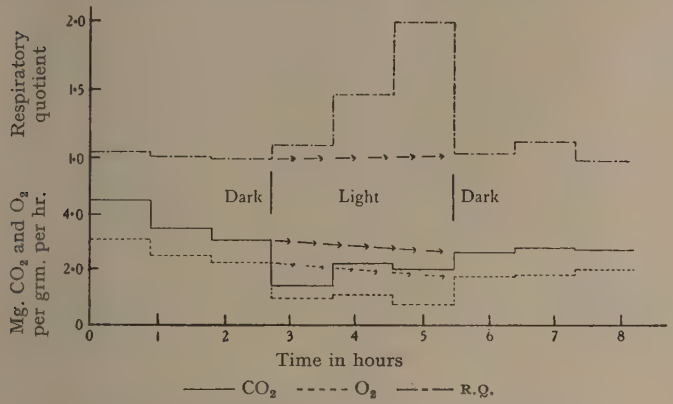



Fig. 13. Effect of light on respiration of *Spirogyra*.

In the first, Fig. 13, the algae were allowed to respire for three periods in darkness and then the light was switched on for another three periods, after which the experiment was again continued for three periods in the dark. The respiration originally started high, 4·45 mg. carbon dioxide and 3·05 mg. oxygen, and then like the typical curve fell steadily up to the end of the third period. Light was now given and the respiration fell off suddenly and remained low, until the light was put out when it again rose. The main point of interest is the R.Q. Originally it was near to unity, but as soon as light was given it rapidly rose so that in the third period the ratio was 2. When again in darkness it immediately fell to unity.

In the next experiment, Fig. 14, a longer exposure to light was given in order to ascertain its influence on the R.Q. The algae were placed in the dark for two periods, then for six periods in light and finally for two periods in the dark again. The graphs obtained are

fundamentally the same as in the last experiment, and the R.Q. shows a maximum of about 2.

In the third experiment, instead of employing artificial light, the

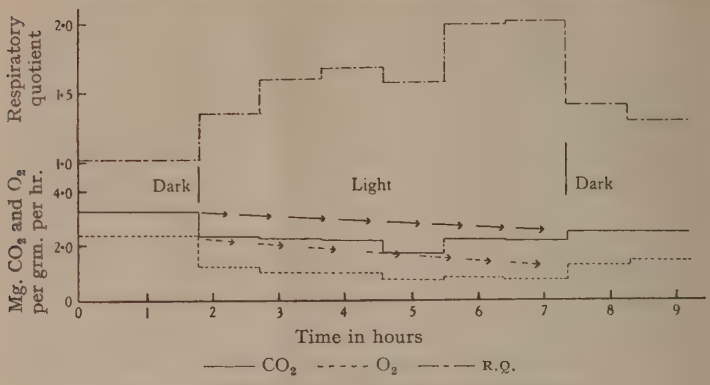



Fig. 14. Effect of light on respiration of *Spirogyra*.

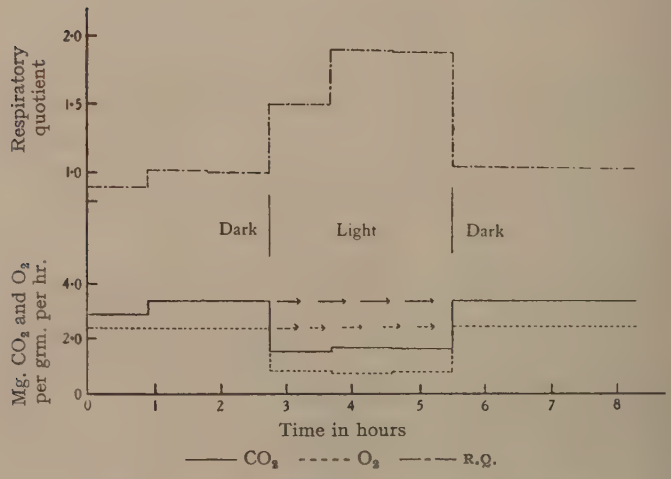



Fig. 15. Effect of sunlight on respiration of *Spirogyra*.

apparatus was taken out in the open and the algae were exposed to sunlight (Fig. 15). The sky was clear and the algae received full midday light. Here also the graph obtained was similar to the previous ones, thereby showing that both artificial light and sunlight have a similar effect on the algae.

In the last experiment, Fig. 16, conjugating filaments were employed instead of normal ones. Almost all the filaments showed formation of tubes, but no passage of protoplasm was seen. Artificial light was employed in this experiment. The carbon dioxide emission started rather low as is characteristic of conjugating filaments. When exposed to light it fell further and kept low throughout the period in light, and again rose when taken back to darkness. The R.Q. in light did not rise to the high values attained in the other experiments, but at first showed a slight fall, and then a rise which kept constant even when the alga was taken back into the dark.

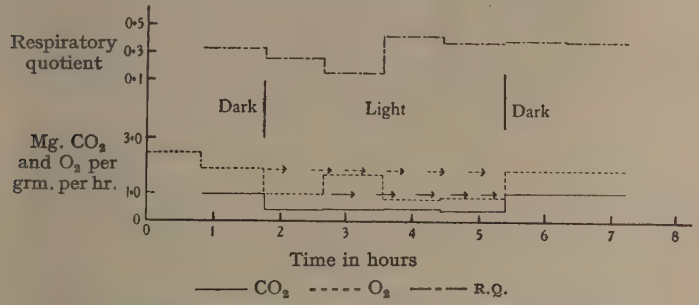



Fig. 16. Effect of light on respiration of conjugating *Spirogyra*.

## B. *Discussion*

### (*a*) *Effect of light on respiration in* Spirogyra.

All the experiments with light show that the carbon dioxide emission and oxygen intake fall greatly on exposure to light. This is surely due to part of the carbon dioxide given out by respiration being assimilated on exposure to light. The lower intake of oxygen is also due to the evolution of the gas in assimilation. It is to be noted here that no extra amount of carbon dioxide was allowed to the plants, and therefore the only source of it was the amount given in respiration. However, before all the gas evolved in respiration is assimilated part of it escapes out into the surrounding medium, and therefore a fair amount of carbon dioxide is detected even on exposure to light.

Regarding the influence of light on respiration, there exists no difference of opinion as to the photosynthetic part of it. Since Borodin (2) showed that after an exposure to light the leafy shoots undergo more vigorous respiration, the indirect influence of light on

plants having chlorophyll has been confirmed by various authors. This is due at any rate in part to the high concentration of sugars formed by photosynthesis.

Regarding the indirect effects of light apart from the photosynthetic one, the results so far obtained are contradictory and probably inconclusive. Thus the experiments of Bonnier and Mangin (1), Elfving (4), and Lowschin (11) showed a somewhat retarding effect of light on respiration. It is to be noted that these experiments were performed with parts of plants devoid of chlorophyll. Maximov (12) found little or no effect of light on respiration.

On the other hand, Meyer and Deleano (13) noted a daily periodicity in respiration under natural conditions. Spoehr (18), from his experiments, came to the conclusion that during the day the respiration of plants increases on account of ionisation of the air brought about by the sun. He suggested that owing to ionisation of the oxygen, autoxidation is instigated in the protoplasm. Middleton (14), with barley seedlings, and Whimster (23), with leaves of *Pelargonium zonale*, have found a great increase of carbon dioxide production in presence of ionised air, the effect being greater in green leaves.

Very recently Ranjan (17), from his experiments with *Croton* leaves, concluded that apart from the photosynthetic influence, light greatly enhances the respiration of leaves. In a paper under publication, he has shown that the enhancing effect is also true of *Pistia* shoots. He, however, found no such effect on *Pistia* roots. This he believes to be due to the lack of anthocyanins in the roots, which are thus unable to absorb the light rays.

Very recently Parija and Saran (16) have found that light accelerates the respiration of *Aralia* sp.

The respiration curves of *Spirogyra* in light do not warrant the conclusion of any marked effect of light on the carbon dioxide emission. When the plants were put back in the dark after an exposure to light, the respiration rose to a level which coincided with the probable intensity of normal *Spirogyra* left in darkness continuously (cf. Fig. 2).

According to Ranjan all plants in which respiration is enhanced by light show a gradual fall in respiration when put in the dark. This feature is entirely absent in the experiments with *Spirogyra*. It is therefore to be concluded that there is no direct effect of light on the respiration of these plants.

Then by joining the respiration values before and after the exposure to light, the probable respiration curve in *Spirogyra* during the period in light can be obtained. The arrow-head lines in Figs. 13–

16 thus represent the true respiration of the filaments during the period. It is to be seen that the true R.Q. due to respiration only remains constant throughout. The rise in the observed R.Q. is to be attributed to some indirect effect of light, the consideration of which will be taken up later.

(*b*) *Assimilation of* Spirogyra *in absence of external supply of carbon dioxide.*

The arrow-head lines in Figs. 13–16 represent the measure of respiration in light. From these and the actual amounts of carbon dioxide given out and oxygen taken in by the plants, the amounts of carbon dioxide used and oxygen given out in assimilation can be ascertained. Table IV shows these calculated amounts. The assimilatory quotient (volume of carbon dioxide/volume of oxygen) is also shown.

TABLE IV

| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|
| Period in light | Actual amount of carbon dioxide given out mg. | Hypothetical respiration mg. | Carbon dioxide assimilated mg. | Actual amount of oxygen mg. | Oxygen taken in for respiration mg. | Oxygen assimilated mg. | Assimilatory quotient |
| | | | *Experiment represented by Fig.* 13 | | | | |
| 1 | 1·42 | 3·0 | 1·58 | 0·93 | 2·1 | 1·16 | 1·02 |
| 2 | 2·19 | 2·8 | 0·61 | 1·11 | 2·0 | 0·89 | 2·0 |
| 3 | 2·04 | 2·7 | 0·66 | 0·75 | 1·9 | 1·15 | 2·4 |
| | | | *Experiment represented by Fig.* 14 | | | | |
| 1 | 3·1 | 3·1 | 0·80 | 1·24 | 2·2 | 0·96 | 1·65 |
| 2 | 2·22 | 2·90 | 0·68 | 1·01 | 2·0 | 0·99 | 2·0 |
| 3 | 2·18 | 2·8 | 0·62 | 0·94 | 1·80 | 0·86 | 1·91 |
| 4 | 1·69 | 2·7 | 1·01 | 0·78 | 1·6 | 0·82 | 1·26 |
| 5 | 2·20 | 2·6 | 0·4 | 0·80 | 1·6 | 0·8 | 2·75 |
| 6 | 2·16 | 2·5 | 0·34 | 0·76 | 1·5 | 0·74 | 2·99 |
| | | | *Experiment represented by Fig.* 15 | | | | |
| 1 | 1·66 | 3·4 | 1·74 | 0·76 | 2·4 | 1·64 | 1·3 |
| 2 | 1·7 | 3·4 | 1·7 | 0·66 | 2·4 | 1·74 | 1·39 |
| 3 | 1·66 | 3·4 | 1·74 | 0·64 | 2·4 | 1·74 | 1·38 |
| | | | *Experiment represented by Fig.* 16 | | | | |
| 1 | 0·35 | 1·0 | 0·65 | 0·91 | 1·84 | 0·93 | 1·97 |
| 2 | 0·45 | 1·0 | 0·55 | 1·7 | 1·85 | 0·15 | 0·38 |
| 3 | 0·47 | 1·0 | 0·53 | 0·79 | 1·86 | 1·06 | 2·72 |
| 4 | 0·45 | 1·0 | 0·55 | 0·82 | 1·87 | 1·03 | 2·59 |

Column 4 of the table gives the amounts of carbon dioxide actually assimilated during the periods in light. This amount is very much less than what is expected. Under favourable conditions assimilation is several times greater than respiration in the same period. As has been said before the cause of this lies in the fact that no external

carbon dioxide was allowed to the plants, and thus only the amount given out in respiration could serve as a source of carbon dioxide for assimilation. The low intensity of assimilation is therefore only due to shortage of carbon dioxide available to the plants.

However, in spite of the fact that the plants had sufficient light, only a portion of the carbon dioxide respired was assimilated; the rest diffused out into the surrounding medium. It is evident that these plants cannot assimilate the carbon dioxide given off in respiration quite so quickly as the gas diffuses off into the surrounding liquid. The relation between the two alternating fates of the carbon dioxide given out in respiration (diffusion and utilisation by photosynthesis) is not constant and seems to be rather obscure, probably due to interaction of some other factors.

(*c*) *Assimilatory quotient and influence of light on oil formation in* Spirogyra.

Fig. 17 gives the assimilatory quotient values from Table IV obtained by calculations in a graphical form. It is considerably

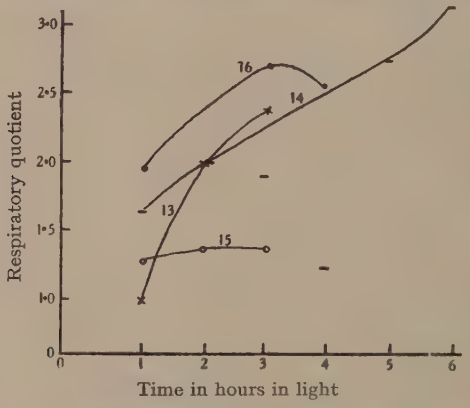



Fig. 17. Assimilatory quotient in *Spirogyra* in water free from carbon dioxide.

greater than the expected value of unity. Such a high oxygen emission immediately suggests the probability of its production in some process other than that of assimilation of carbon dioxide and water to sugar.

The appearance of oil and the failure to obtain R.Q. values greater than unity at any stage in the respiration experiments can now be

correlated with the high evolution of oxygen in light. It is quite possible that the extra amount of oxygen given out in light experiments is due to its evolution in formation of oil from sugar. The oil found in *Spirogyra* thus begins to form at a stage previous to conjugation, and, as would be evident from the assimilatory quotient of conjugating *Spirogyra*, the process continues vigorously till the end.

This reaction, however, takes place only in light. Thus in the dark experiments the R.Q. was never greater than unity. In absence of light, the reverse process of oxidation of fats begins and consequently a great amount of oxygen is taken in:

$$19C_6H_{12}O_6 \underset{\text{dark}}{\overset{\text{light}}{\rightleftharpoons}} 2C_{57}H_{104}O_9 + 10H_2O + 43O_2.$$

Ivanow (9) has shown that lipase can either hydrolyse a fat or may synthesise it from fatty acid and glycerol. He found that if a glycerol extract of seed be mixed with oleic acid, fat is synthesised, while it is again split up on diluting with water.

No influence of light on the reversibility of lipasatic action is as yet known, but the peculiar oil metabolism strongly suggests such an influence.

It is to be noted here that in no light experiment was the normal assimilatory quotient of unity obtained. There can, however, be no doubt that in young vegetative filaments a value very near unity would be obtained. In the season when these experiments were carried out no young and rapidly growing filaments could be procured to verify this assumption. But the rather low assimilatory quotient values in the experiment with sunlight show such a possibility. Probably in this case the condition of the filaments was not so advanced as in the other cases. Another fact to be noted in this connection is that the unfavourable laboratory conditions, the high temperature and the starting of experiments in pure water hasten the conjugation stages, and the normal conditions are difficult to obtain.

(*d*) *Influence of light on conjugation in* Spirogyra.

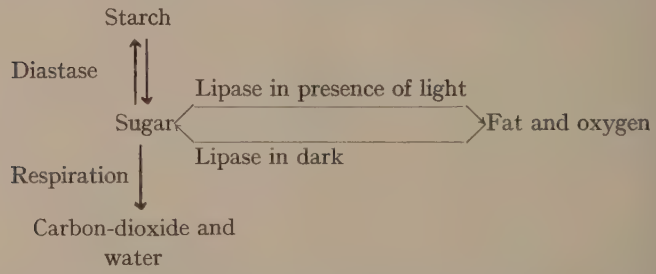
The importance of light on the formation of oil in *Spirogyra* has been shown under the last head, and now it is important to know whether light has any influence on the process of conjugation itself. The following experiment was performed in order to have an idea of this problem.

Several samples of normal *Spirogyra* were taken in different dishes containing pond water, distilled water and 0·05 per cent. sodium bicarbonate solution. One set of these was kept in a corner in the laboratory in diffused light, while the other was in the open in sunlight. Though the filaments in most of the cases in light showed beak formation within 2–7 days, in no case of the other set was any stage of conjugation seen. After about a week and a half these filaments died. Thus even in unfavourable circumstances when the vegetative filaments cannot thrive the sexual stages are inhibited by weak light.

Thus strong light is essential for conjugation in *Spirogyra*. Recently Garnar and Allard (6), Hendricks and Harvey (8), Wann (21) and others have found that light has a marked influence on the reproduction and blooming of various plants. The cause of this has not yet been definitely ascertained, and the various views seem to be speculative. The experiments with *Spirogyra* do not give any clue to this problem, but it is seen that light influences the formation of oil, which is intimately connected with the process of conjugation.

(*e*) *Economics of oil metabolism in* Spirogyra.

As a result of the discussions given above the following scheme is suggested to represent the reactions in conjugating *Spirogyra*:




As the conjugation stages advance, more and more of the reserve starch gets converted to fat; so that in the final stage when the zygospore is fully formed it contains only fats. The fat in the zygospore is used as a reserve food during germination, when it is again converted to carbohydrates. In higher plants also many seeds contain oily reserve matters. The chief advantages therefrom are that only a small space is required for the storage (for the carbohydrates of the same energy content have a greater bulk), and that the storage of oil minimises the danger of draught.

The conversion of food matters to oil is thus of great importance

to the plants. The question now arises as to the special advantage of the reversibility of the reaction in the dark in *Spirogyra*.

The plants in these stages, as has been shown before, require a considerable amount of free energy. At the same time, however, they also require a good accumulation of food reserve to be used during the germination of the zygospores. Conjugation and other sexual processes in the algae are only adaptations to tide over some unfavourable conditions when vegetative growth becomes impossible. Shortage of food material is very often the cause of the beginning of the sexual processes. Thus in these stages the filaments have great difficulty in supplying enough food matter for respiration purposes without greatly minimising the food reserve in zygospores.

The peculiar oil metabolism in *Spirogyra* is the instrument by which the loss of food matter in respiration during darkness is minimised. During darkness, instead of deriving energy from the oxidation of sugars and thereby releasing the carbon dioxide thus evolved, part of the energy required is obtained by conversion of oil to sugar. In light the sugar so formed and the reserve carbohydrates are again converted to oil, the light energy being used for the purpose. Thus without any great loss of carbohydrate food an adequate supply of energy is achieved.

## Summary

A simple apparatus, being an improvement on the adaptation of Winkler's method by Osterhout and Haas, for estimating the respiratory and photosynthetic activities in water organisms has been described.

It has been found that the evolution of carbon dioxide in normal *Spirogyra* and *Sirogonium* is comparatively high and the respiratory quotient equal to unity, but as the sexual stages progress, the carbon dioxide emission and the respiratory quotient gradually fall to very low values. The intake of oxygen, however, shows at first a rise and then a lesser fall.

It has also been found that though purely vegetative filaments do not contain any oily matter, a great number of oil globules appear in the filaments undergoing conjugation. Starch is abundant at all times except in the very latest stages of zygospore formation, when it is completely converted to fats and oils.

It has been suggested that the very low respiratory quotient in conjugating *Spirogyra* and *Sirogonium* is due to a very rapid oxida-

tion of fats and oils to carbohydrates. This necessitates the fixing of a great amount of oxygen without any liberation of carbon dioxide.

As the oxidation of oils to carbohydrates liberates a considerable amount of free energy, the evolution of carbon dioxide in conjugating *Spirogyra* cannot be used as an index of the metabolic activity of the organisms. On the other hand, this purpose can fairly be served by the intake of oxygen, for the energy liberated per gram of oxygen used for oxidation of sugars is approximately equal to the energy released per gram of oxygen used for oxidation of fats to carbohydrates.

The metabolic activity of the conjugating *Spirogyra* increases, as evidenced by the oxygen intake, in the first stages of conjugation, and then it falls to about half the value of normal *Spirogyra*.

Light is of great importance to conjugating *Spirogyra*. It is only in light that the conversion of carbohydrates to fats and oils is possible, the light energy being used for the purpose. In the absence of light, the reverse process of oxidation of oils to carbohydrates takes place. Conjugation fails to take place in the absence of light.

The conversion of carbohydrates to oils in light and the reverse process in dark are of great economic importance to the organisms. This process enables the filaments to prevent the loss of carbon while respiring in the dark.

The writer wishes to express grateful appreciation to Dr S. Ranjan for helpful suggestions and criticisms and for his interest in the progress of this study.

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# THE SPORE DISCHARGE MECHANISM IN *BASIDIOBOLUS RANARUM*

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(With 2 figures in the text)

The life history of *Basidiobolus ranarum* has been considered in detail by Eidam (1) in 1886 and by Levisohn (2) in 1927. Eidam describes violent spore discharge involving the projection of the conidium together with the "basidium," the two separating in mid-air. The mechanism of the discharge does not, however, appear to have been appreciated. It is proposed in this note to describe the rocket mechanism which is apparently responsible for the propulsion of the conidium to a distance of 1–2 cm. This was discovered while examining material of *Basidiobolus* in order to compare it with *Pilobolus*, a fungus which, superficially, it resembles very closely.

*Basidiobolus ranarum* in the conidial condition can usually be obtained by collecting the excrement of frogs and keeping it on moist filter paper in a Petri dish.

In spite of the striking general resemblance between the mature sporangiophore of *Basidiobolus* and *Pilobolus* the method of discharge in these two fungi is very different. In *Pilobolus* the turgid subsporangial bulb ruptures along a definite line of weakness just below the junction between the sporangium and the columella. The stretched elastic wall of the sporangiophore *below* this dehiscence line immediately contracts squirting out from the subsporangial bulb a drop of sap which carries the sporangium with it. Up to a point the arrangement in *Basidiobolus* is similar. In the subconidial bulb there is a definite line of weakness where dehiscence takes place, but this line of weakness occurs towards the base of the subconidial bulb and not towards the apex as in *Pilobolus*. Further, the elastic region of the wall is *above* this line of dehiscence, not below it.

Mature conidiophores were observed on the excrement of frogs under the low power of the microscope and discharge was watched. As is to be expected discharge takes place so rapidly that no stages in the process can be observed. At one moment the conidiophore appears straight and turgid, the next nothing is to be seen either of

the conidium and its "basidium" or of the remainder of the conidiophore. The appearance of the conidiophore just before discharge is illustrated in Fig. 1 A. The incipient line of dehiscence can be seen, due to the different reflecting powers of the "basidium" and the lower region of the conidiophore and also on account of the somewhat thicker wall of the "basidium." The thicker nature of this wall is due to the fact that it is double in contrast to the single wall composing the remainder of the conidiophore. The discharged conidium is thrown to a horizontal distance of 1–2 cm. The "basidium" is usually thrown about 0·5 cm.

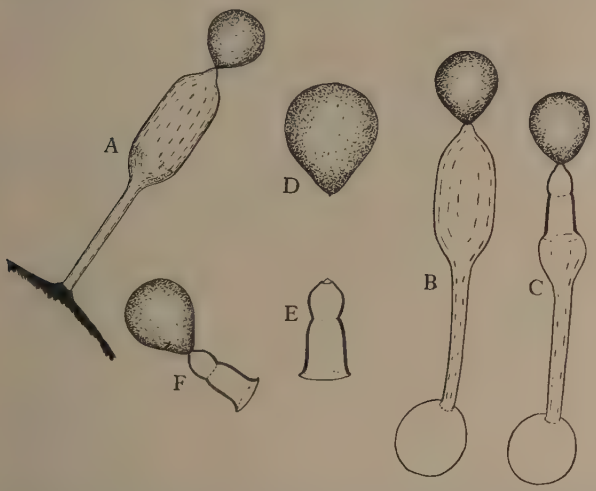


Fig. 1. *Basidiobolus ranarum*. A, mature conidiophore growing on natural substratum; B, living conidiophore mounted in water; C, the same after the addition of a trace of iodine; D, a discharged conidium; E, a discharged "basidium"; F, a conidium and "basidium" which have failed to separate in mid-air. A, B and C × 380; D, E and F × 480.

Fig. 1 B shows a mature conidiophore, derived directly from the germination of a conidium, mounted in a drop of water. Fig. 1 C shows the same conidiophore after being killed by the addition of a trace of iodine. It is to be noted that it is the "basidium" which is the elastic region of the conidiophore wall; the lower region of the conidiophore undergoes no shrinkage on death. This gives the clue to the probable mechanism of discharge. The pressure in the conidiophore becomes so great that rupture occurs along the line of dehiscence below the "basidium." Then the elastic wall of the "basidium" suddenly

contracts; it squirts sap backwards, flies off on the recoil and carries the conidium away with it. The mechanism of discharge resembles that of a rocket. In the air, as described by Eidam, the "basidium" (Fig. 1 E) becomes separated from the conidium (Fig. 1 D) by the rounding off of the conidium where the point of the "basidium" pushes into it. Very occasionally this separation fails to occur (Fig. 1 F).

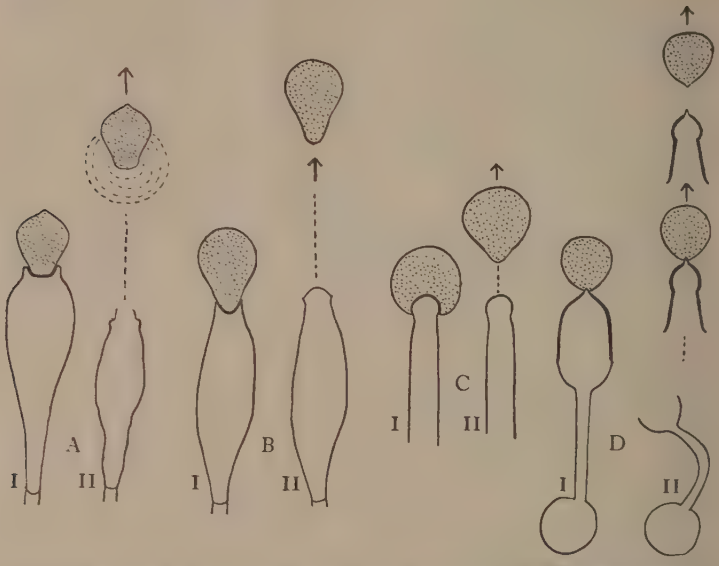


Fig. 2. Diagrams to illustrate types of spore discharge in the *Entomophthorales*. A, *Empusa muscae*; B, *E. Grylli*; C, *Conidiobolus villosus*; D, *Basidiobolus ranarum*. I before, II at discharge. A and B based on figures and description by Thaxter(4), C on those of Martin(3).

It is of interest to compare the method of discharge in *Basidiobolus* (Fig. 2 D) with that found in the other genera of the *Entomophthorales*. In this order of the *Fungi* violent spore discharge is the rule, being exhibited by all genera except *Massospora*. In *Empusa muscae* (Fig. 2 A) the turgid conidiophore explodes (Thaxter(4)) discharging the conidium together with a drop of conidiophore sap, much after the fashion of *Pilobolus*. In *Empusa Grylli* (Thaxter(4)) the columella is pushed back into the conidiophore by the bulging wall of the spore. On discharge this re-entrant columella is suddenly pushed out by the turgidity of the conidiophore and the conidium is thrown off (Fig. 2 B). In *Conidiobolus villosus*(3) the columella pushes into the conidium.

The spore in this case suddenly rounds off and in doing so bounces away from the conidiophore (Fig. 2 C). It is clear that in the *Entomophthorales* violent spore projection has been evolved along more than one line of development.

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# POLLEN ANALYSIS. AN OUTLINE OF THE PROBLEMS AND POTENTIALITIES OF THE METHOD

## PART I. TECHNIQUE AND INTERPRETATION

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(With 9 figures in the text)

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### Introduction

The first aim of pollen analysis is to establish the pollen content of recent geological deposits. This pollen flora has been derived from surrounding vegetation, especially the wind-pollinated plants, during formation of the deposit. The deposits vary greatly in the extent to which they preserve the pollen, but in the waterlogged anaerobic layers of growing peat beds the pollen membranes are extremely well preserved, and after removal of humic material by alkali or acid oxidation can be readily identified. A very large component of the pollen rain incorporated in these deposits has always been the pollen of the forest trees, for these are anemophilous and over large areas are natural climatic dominants. Pollen analysis has been mainly concerned with the tree pollen however, not only on account of its abundance, but because as vegetation dominants the trees are the most direct indices to past climates, and also as dominants of the natural vegetation must have played a great part in the lives of prehistoric people (14).

Not only are the pollen grains evidence of the former presence of different kinds of trees but counts can establish indices to the relative frequency of different tree genera. "By establishment of these frequency figures layer by layer through the pollen-bearing strata it becomes possible to follow former plant-geographic changes from place to place and from one time period to another" (von Post(27)).

In the vertical sequences through the deposits of a district it is possible to establish fundamental points of similarity and by these in turn to establish horizons of equal age.

Such methods of analysis have been applied both to inter-Glacial(19, 33, 21) and post-Glacial deposits, but it is for the latter particularly that the last twenty years have seen intensive investigation in many parts of the world. Since the pioneer work of von Post it has become increasingly clear that in broad outline the same story of forest movement is recorded from the post-Glacial deposits of all Europe. Extension of the wide body of pollen analysis data continues to confirm von Post's threefold division of post-Glacial time on a climatic basis into (*a*) the period of increasing warmth, (*b*) the period of maximum warmth, (*c*) the period of decreasing warmth. This climatic sequence is strongly indicated by the forest movements. In the period of increasing warmth following the last retreat of the ice-sheets forests of pine and birch advanced northwards across Europe. After them followed the warmth-loving trees such as oak, elm and lime, and during the warmth maximum these trees reached their greatest extent and importance. Towards the end of the period of maximal warmth the beech and hornbeam spread extensively, but, together with the components of the mixed oak forests, showed retrogression in area and abundance in the following period of diminishing warmth, in which the Conifers showed a tendency to return to their former dominance. This story is clearly evident in the small pollen diagram (Fig. 1, after Keller(20)), from central Switzerland. This shows also the marked hazel maximum which occurs over a very large part of Europe at the transition to the period of maximal warmth. Naturally there are great differences in the expression of this forest succession between the north and south of Europe, and some, though smaller ones, between east and west. These general drifts are briefly mentioned in a later section of this paper, but it is not the present aim of the author to collect and analyse the very large mass of data acquired all over Europe by pollen-analysis methods. Despite careful attention to theoretical and practical problems of the methods of pollen analysis by numerous workers in the subject and a

recognition of the extreme caution necessary in interpreting the results it yields, there is no simple general statement of the precise scope and limitations of the method. It is this alone which is intended in the following pages; and though not exhaustive of even this more limited subject, it will present a general picture of what the method can and cannot do, and what technique is likely to be of most profit in developing an intensive study of the British post-Glacial deposits.

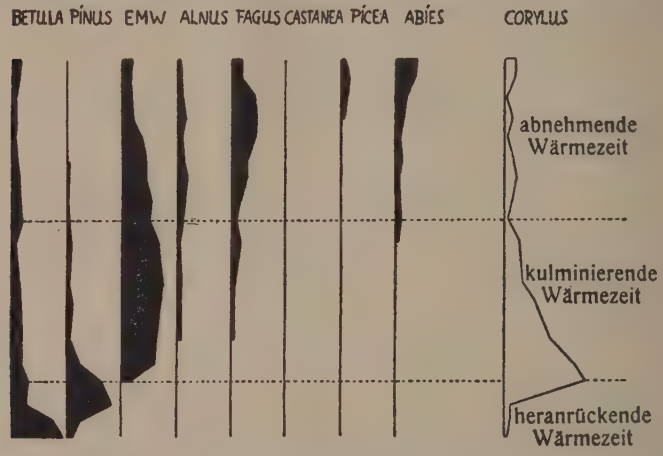


Fig. 1. Diagram from Keller for Switzerland showing division of the post-Glacial into three climatic periods, and the corresponding changes in pollen composition showing altering forest composition. In each vertical column is shown the pollen of a single tree genus expressed as a percentage of the total tree pollen. *Corylus* is also expressed as a percentage of this figure, but does not contribute to it. EMW stands for "Eichenmischwald," the mixed oak forest with *Quercus*, *Ulmus* and *Tilia*.

## Preparation of samples

The usual method of preparing peat samples for analysis is based on treatment with hot alkali. Calcareous marls are treated with hydrochloric acid, and the organic component in siliceous sediments is concentrated by decantation and by treatment with hydrofluoric acid. The alkali treatment of peat is extremely simple: in most cases a small amount of the sample is boiled up on a microscope slide with a few drops of 10 per cent. KOH or NaOH. The macerated material is then examined directly, or it may first be washed and afterwards mounted in glycerine jelly, either unstained or coloured with safranine. Various modifications of this simple technique have been described, mostly with a view to placing the pollen counts on an

absolute basis; a given weight or volume of peat is boiled up with alkali, is repeatedly washed, centrifuged, and finally made up in such manner that a known amount is present on each slide.

More recently G. and H. Erdtman (9) have proposed an alternative maceration method based on mild oxidation followed by acid hydrolysis. The oxidation agent is $NaClO_3$ in a mixture of acetic and sulphuric acids. It is allowed to act for some hours in the cold and then the residue is washed and dried with acetone and ether. The mass of material so produced is treated with 80 per cent. sulphuric acid which hydrolyses the abundant polysaccharide fraction, leaving a sample extremely rich in pollen grains. This residue is made up in lacto-phenol stained with methylene blue, and a definite volume is placed for counting in a specially made chamber of the type of a blood-corpuscle counter. The authors claim that this method, though yielding results like those given by the alkali method, concentrates the pollen so much more, that analyses can be made of samples far too poor in pollen for alkali preparation.

In the same publication attention is drawn to a common source of error in the final mounting of material. It is shown that pressure on the cover-slip causes over-representation of large pollen grains by the squashing out of smaller grains at the edges.

The same authors show that prolonged alkali treatment causes destruction of the pollen-grain membranes, and suggest that this destruction is differential for different tree genera. Though this is probably so, the evidence suggests on the whole remarkable uniformity in pollen composition up to the final disappearance of all the grains, and it is clear that further evidence on this point is desirable, and that it should be given as results which have been subjected to statistical proof.

## Identification

The identification of fossil pollen is based on criteria of size and shape of the grain, number, size, form and arrangement of the germ pores, thickness of the pollen-grain membranes and surface markings or thickenings of various kinds. The genera of British trees which are preserved in peat have pollen grains now so familiar in appearance that it is unnecessary to give any key to their identification, although Fig. 2 has been included so as to show their salient characteristics when seen at different angles and when drawn on a common scale.

There is only one serious possibility of confusion between the different genera. The pollen grains of *Salix* and *Fraxinus* are very

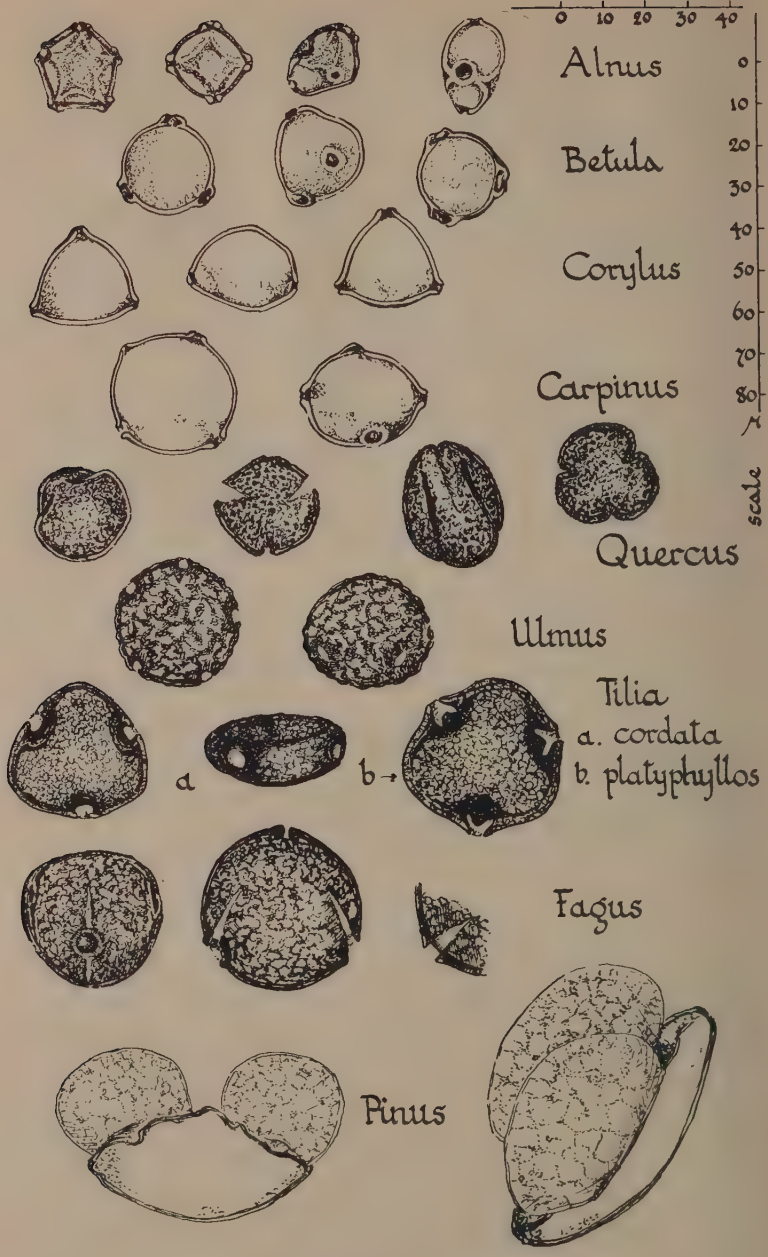


Fig. 2. Diagram to show the salient characteristics of the pollen of the chief genera of trees which are represented in British post-Glacial deposits. The grains are shewn as they appear in the subfossil state, they are all drawn to the same scale, and views from different angles are presented so as to give an idea of the solid shape of each grain.

similar to one another in appearance, and though they seem to differ in size the author is not aware of any studies of the size variation in pollen from *Fraxinus excelsior* nor from the numerous species of *Salix* which might be supposed to have left pollen in British post-Glacial deposits. It would seem that until these studies have been made the only means of separating the two genera is to use the minor morphological character suggested by Meinke (25) of the clearer longitudinal folds in *Fraxinus* and to adopt a provisional size limit of about 27 $\mu$ for length of the grains, regarding the larger ones as *Fraxinus* and the smaller as *Salix*. It is to be noted that the pollen of *Fraxinus* is frequently said not to be preserved; so that the *Salix-Fraxinus* type of fossil grain is for the time being perhaps best spoken of as "salicoid." Because of this uncertainty of identification, coupled with the fruticose habit of many Salices, it is not customary to reckon salicoid grains in the total counts of tree pollen, and for this reason also we omit illustration of the grains in Fig. 2. Though the tree pollens are unlikely to be confused with one another yet some of the genera have pollen extremely like that of various shrubs or herbaceous plants to which they may be quite unrelated. Thus the pollen of *Quercus* is not distinguished from that of *Viola*, though there is seldom any doubt that where the pollen is present in quantity and in deposits found some distance from forest sites, the pollen is that of the wind-pollinated tree. Similarly pollen of *Fagus* is indistinguishable from that of *Hippophae*. Since *Hippophae rhamnoides* is not confined to coastal habitats, but appears widely on scree and moraine soils on the continent, there is always the possibility that records of "beech" grains in deposits greatly pre-dating the general beech invasion of a region, may, in fact, be due to *Hippophae*. This is the interpretation given by Thomson (34) to explain the presence of such grains in the subarctic layers of peat deposits in Esthonia, a country which has never been reached by beech forests in the post-Glacial period. The salicoid type of grain is much like that of *Adoxa*, and *Corylus* pollen closely resembles that of the nettle, *Urtica dioica*. Under conditions of prehistoric forest dominance these herbaceous plants probably were less likely to affect the general tree-pollen deposition than under present-day conditions of extremely extensive forest clearing. It is sometimes a matter of difficulty to distinguish between grains of *Corylus* and *Betula*; the usual criteria of distinction lie in the germ-pore characters shown in Fig. 2, the sharply projecting thick ring round the uniform wide pore of *Betula* contrasting with the gradual and slight thickening which surrounds the conical and narrower pore of *Corylus*.

Nevertheless, a small percentage of grains sometimes appear to be intermediate in respect of these characters.

A much more serious possibility of error than this lies in the possibility of confusing pollen of the shrubs, *Corylus* and *Myrica*. It used to be generally held that the pollen of *Myrica* was seldom preserved. Madame Szafer (18), who has been able to work out criteria for distinguishing the two types of grain, says that there is no evidence to suggest that this is so. The chief distinguishing characters are: (1) *Myrica* has a thicker exine; (2) the exine thickens near the pores in *Myrica* whilst it is equally thick in *Corylus*; (3) there is only one thick layer in the exine of *Myrica* but there are two in *Corylus* (besides other thin layers); (4) in *Myrica*, on the inner surface of the exine and extending a short way in from each pore, the thickening is visible in optical section as a bright area on the wall.

Though Madame Szafer's paper was published in 1928 the problem of recognising separately the pollen of *Corylus* and *Myrica* is unrecognised in subsequent pollen analysis papers; this is partly due, no doubt, to the difficulty of distinguishing the two pollens and partly to the continued belief that *Myrica* pollen is easily destroyed. Fortunately the "coryloid" pollen is not reckoned in total tree pollen, but in view of the importance of the boreal hazel maxima (8) and in view of the widely different edaphic requirements of *Corylus* and *Myrica* and therefore of the inferences to be drawn from abundant pollen of either of them, it is very undesirable that the problem should be left at this stage.

Though the tree *genera* are separable by their pollen, in most cases the *species* are not. Thus *Quercus robur* and *Q. sessiliflora*, though probably differing in size, are otherwise alike, and there is no means of distinguishing the pollen of the separate species of *Betula*, *Salix* or *Ulmus*, nor do the systematic relationships within these genera suggest that such a means is likely to occur. The size variation of the *Betula* species has been worked out by Madame Szafer and others, and Fig. 3 gives some idea of the range involved. This is a diagram by Bertsch in which the thick line shows the size of fossil pollen from the birch phase of the Federsee pollen diagrams. From the form of this curve Bertsch concludes that *Betula nana*, *B. pubescens* and *B. verrucosa* were all present in this area and contributed to the birch pollen maximum. *B. humilis* may also have been present but could hardly have alone accounted for the major peak of the fossil pollen size curve. Since only one species of *Alnus* occurs in Britain the pollen is unlikely to be attributable to others, but it should be remembered

when comparing British pollen analyses with continental results, that in the latter *Alnus* pollen includes not only that of *A. rotundifolia* but also of *A. incana* and *A. viridis*, which are indistinguishable from it.

In the same way, although it has been shown that the pollen of different species of pine are distinguishable from one another (12–15), the total pine pollen curve in most continental diagrams includes the pollen of *Pinus montana* and *P. cembra* as well as *P. sylvestris*. Erdtman suggests (7) that it is partly on account of *P. montana* pollen that the pine maximum in many parts of the continent falls so much earlier than in England (i.e. in the Early Boreal or pre-Boreal). Care is

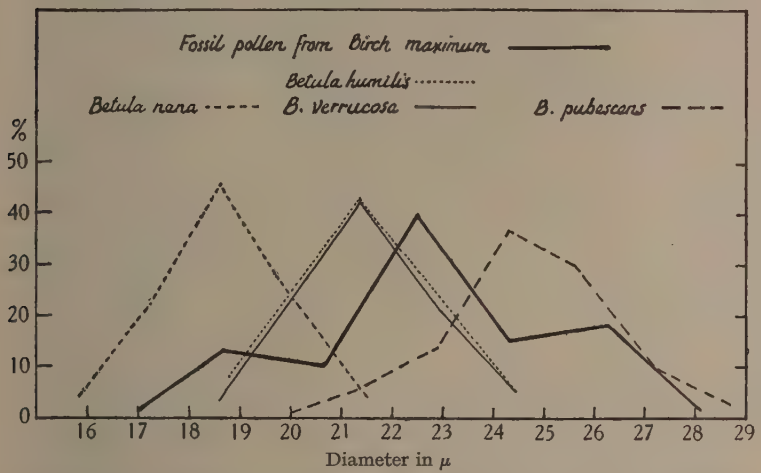


Fig. 3. Size variation curves for recent pollen of species of *Betula* compared with a similar curve for fossil birch pollen from the birch maximum in the Federsee region. After Bertsch(3).

therefore necessary in comparing the British pine pollen curves, due solely to *P. sylvestris*, with those of the continent.

In the case of *Tilia* alone of the British tree genera is it possible to distinguish the species. It has been shown by Trela (35) that *T. cordata* Mill. and *T. platyphyllos* Scop. have pollen which can be readily distinguished by the following criteria. The grains of *T. cordata* are triangular in outline, the germ pores lying in the middle of the sides, but *T. platyphyllos* has hexagonal grains with the germ pores at alternate corners. The grains of the latter alone show the cutine projecting as a conspicuous peg through the base of the germ-pore cavity. Finally Trela gives the distribution curves for the diameters of the grains of the two species, both recent and fossil (Fig. 4). Our

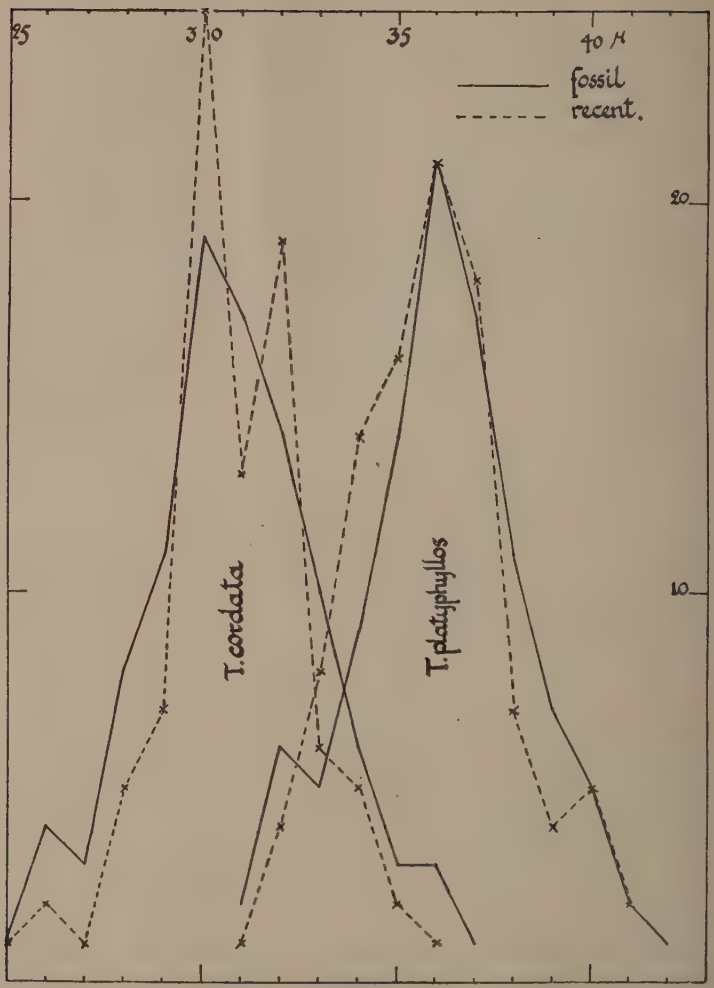


Fig. 4. Size variation curves for recent and fossil pollen of *Tilia cordata* and *T. platyphyllos*, after Trela(35). Based on measurements of samples of 100.

own preparations have shown that *T. europea*, which is usually considered a hybrid between *T. cordata* and *T. platyphyllos*, has pollen grains of intermediate morphological character, and, as will be seen from the graph of Fig. 5, also of intermediate size. The respective modes and ranges for the curves are:

| | Range in | | Mode in | |
|---|---|---|---|---|
| | Recent | Fossil | Recent | Fossil |
| *Tilia cordata* | 25–37 | 25–37 | 31 | 31 |
| *T. platyphyllos* | 31–41 | 31–42 | 36 | 36 |
| *T. europea* | 28–42 | — | 34·5 | — |

In the fen and coastal deposits of East Anglia *T. platyphyllos* and *T. europea* are almost entirely absent, but pollen referable to *T. cordata* on morphological grounds is locally quite abundant, and, as will be seen from the curve in Fig. 5, it has a modal size value of 27–30 $\mu$.

It should be noted that the type and media of maceration and mounting have been shown to affect the size of the grains, and such a factor may be partly responsible for the differences in modal size in *T. cordata* pollen from Poland (Trela) and from the fenland peats. The discrepancy is so large, however, that it clearly shows the desirability of wider information as to the factors which may affect the size of pollen grains, not only factors of technique and preparation but the effects of phenotypic and genotypic factors acting through the parent trees.

The identification of spores and pollen grains of plants other than trees almost always accompanies and reinforces the routine estimation of tree pollen. In this task the pollen atlas and key prepared by Meinke(25) for German bog plants is of great value, though each investigator will naturally build up for himself a type collection of recent pollen and spores. The non-tree pollen is of particular interest as key to the successional development of a bog. Some large families such as Cyperaceae and Gramineae have grains which cannot be separated into species except by size, and since each family has species which occur over an extremely wide range of habitats, their pollen has no great indicator value. In this respect the tetrads of the Ericaceae are much more valuable, since the family is on the whole restricted to acid soils. Overbeck has recently added also a systematic comparison of the grains of the middle and north European species of Ericales(26).

Though valuable as indices to local conditions, the non-tree pollens have for obvious reasons far less general use as climatic or chronological indices than those of the trees.

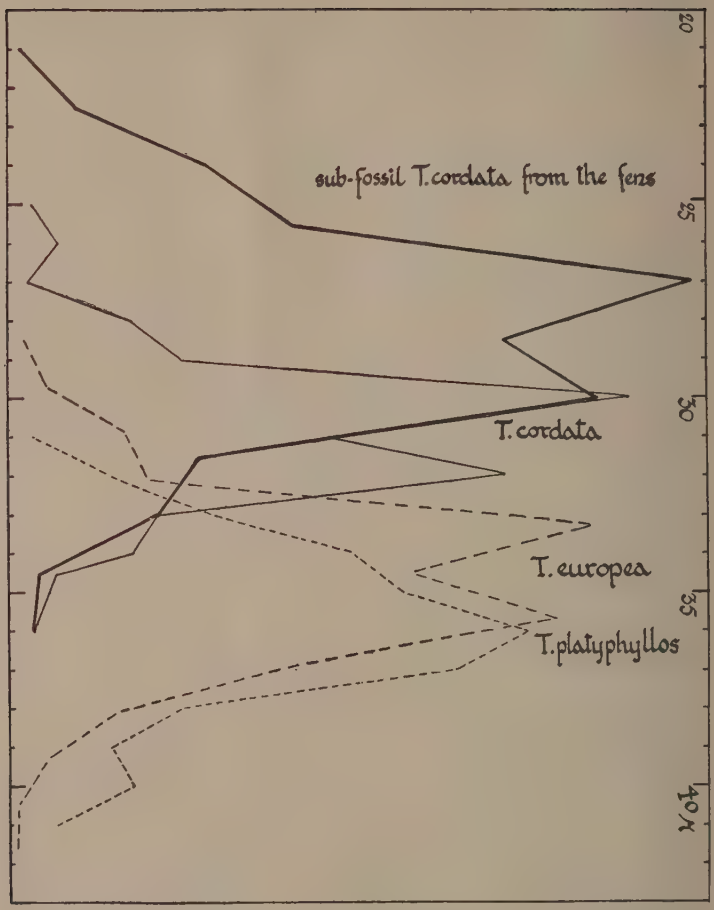


Fig. 5. Size variation curves for sub-fossil pollen of *Tilia cordata* from post-Glacial deposits of East Anglia, from measurements of 431 grains. For comparison size variation curves are given for recent pollen of *T. cordata* and *T. platyphyllos* (after Trela), and of *T. europea*. The latter curve is based on measurements of 302 grains taken from a mixture of the pollen of twelve separate trees.

### Differential pollen preservation

The processes involved in pollen decay are presumed to be both bacterial action and direct oxidation. Pollen is best preserved in waterlogged and unaerated deposits both of mineral and organic origin. In the former class the clays and fine silts often contain well-preserved pollen though identification of the grains is often made difficult by the mineral debris. In coarser material preservation is poor and identification generally impossible. It is in peat that preservation is most complete, though the acid peats are far better in this respect than the alkaline peats of topogenous bogs such as those which characterise the fens and broadland of East Anglia.

Considerable differences exist between different tree pollens in their capacity to resist destruction. Pollen of *Populus*, *Taxus*, *Juniperus* and members of the Rosaceae is easily destroyed, but fortunately that of all the forest dominants is relatively well preserved. Some differences in destructibility may well exist between these, but it is difficult to see how such differences can be evaluated. Over certain genera opinion is conflicting; thus *Fraxinus*, *Myrica* and *Acer* are sometimes quoted as having no pollen preserved in peat, whilst they appear in the analyses of other workers. *Larix* pollen is rather readily destroyed and it appears chiefly in horizons where macro remains such as stubs and cones lead one to think that *Larix* must have had great local density; even in these places the percentage of larch pollen is quite small (Firbas (10)). Hesmer (15) has also suggested that the pollen of *Quercus* is more susceptible to decay than that of other tree genera.

In samples which have suffered much decay it seems very probable that even if destruction affects all the grains equally, the analyses will favour those genera with very readily recognisable pollen, such as *Alnus*, at the expense of other genera such as *Quercus*, which in a crumpled or fragmentary condition are much more difficult to recognise.

### Calculation and presentation of results

From the commencement of pollen analytic investigation it has been customary to count, in all but the most difficult samples, a minimum number of 150 grains of tree pollen. Pollen of *Corylus-Myrica* type is not reckoned in this total. It has been demonstrated that 150 is the minimum number necessary to reduce sampling error to sufficiently below the magnitudes of changes in pollen composition

it is desired to measure, and some statistical observations apparently support this view(2).

The amounts of pollen of different tree genera are expressed as percentages of the total tree pollen, and the *Corylus-Myrica* pollen is expressed also as a percentage of this total. It is a method of obvious disadvantages, since a real variation in the pollen rain in respect of a single genus must always affect the percentage values for all the others. For this reason it would be much preferable to express the amount of each pollen type on an absolute scale. The difficulties in achieving this are quite insuperable, however. Fresh weight or volume bases are excluded by the extremely high and variable water content of peat samples. Dry weight, ash or carbon content, which might at first glance seem suitable, are also unsuitable since they are all affected by variable plant composition, by varying rate of peat growth and varying rates of subsequent destruction. The fundamental absolute value it is desired to investigate must be the pollen rain (or rather pollen incorporation) for each type *per unit time*, but the variations in rate of formation, in material of formation and in degree of subsequent decay and compacting stand in the way of obtaining any present indices to this absolute scale. It is not even permissible to assume that the same net rate of peat formation is maintained in any single peat bog, since the processes of vegetational succession themselves (to say nothing of external climatic or geological factors) will cause changing rates of peat formation vertically throughout the bog, as do the vegetational zonations horizontally. The presence of tussocks and hollows and varying types of plant cover make it probable that the rate of peat formation will even vary foot by foot across the surface of a bog.

Though absolute pollen frequency is liable to such variation within adjacent samples that it is not practicable to express the amounts of pollen of separate tree genera in absolute terms, yet rough estimates of absolute tree-pollen content may in some cases be usefully made. Such estimates frequently are indicative of changing edaphic conditions through the development of a bog; thus in the development of peat bed E, at St German's, a much lowered pollen frequency was closely associated with the development of a peat bed into a phase of fen oakwood when the relatively dry conditions of the forest floor might be supposed adequate reason for the intense destruction of pollen and low resultant frequencies. Absolute frequency of total tree pollen based on number of tree pollen per square centimetre of slide preparation surface was used by Erdtman in his

investigations of northern Scotland and the Scotch isles (6). It gave the results shown in Fig. 6 and appears to demonstrate the possibility of a general diminution of past tree cover progressively across the area towards the Atlantic.

Though absolute pollen frequencies for the separate trees are excluded it might still be supposed possible to estimate the variation in percentage composition due to sampling within a series of samples of the same age. It is, however, almost impossible to trace lateral bedding in most peat beds with sufficient accuracy to guarantee the detection of a layer of uniform age, and from what has already been said with respect to local variation in peat growth it will be clear that neither a horizontal plane nor a plane parallel to the base of the bog

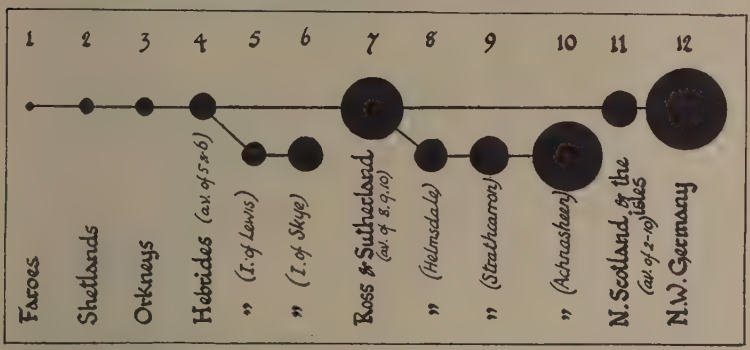


Fig. 6. Diagram after Erdtman(6) showing the diminution in absolute tree-pollen frequency (reckoned as number per unit area of slide surface) north and west from the continent across northern Scotland.

will be quite satisfactory as a surface of contemporaneous peat formation.

An interesting series of observations might be made by exposing a suitable vertical surface in a thick peat bed and by subjecting to analysis samples taken in a close vertical series along each of three or four lines upon the exposed face. By the normal process of analysis similar sequences would become evident in all the vertical series, and these could be used as a rough basis for the establishment on the peat face of lines of equal age. By returning to the protected peat surface with the original sampling points still recorded clearly *in situ* upon it, it should then be possible to secure, along such lines of equal age, samples giving a general knowledge of the variation of tree-pollen content in space across a peat bed. Naturally in such a case it would be necessary to use the greatest care in interpreting the drift of the

pollen curves of the initial vertical series, having in mind as far as possible the whole story of the development of the peat deposit. It is likely that only in a few special cases would peat bogs be susceptible to such experiment, and it seems impossible in the majority of cases to escape from the necessity of expressing the amounts of different tree pollens as percentages of the whole. The chief drawback of this method of expressing pollen analysis results is that there can be no direct indication of the absolute change through a peat bed of the amounts of the pollen of any single tree genus. That is to say, it excludes by implication any direct picture of the changes of absolute density of oak or any other trees growing in the forests of the region throughout the period of formation of the peat. Nevertheless the method will always indicate changes in the *relative* abundance of the pollen of different tree genera. Thus, if oak increases and alder decreases during the formation of a peat deposit, this change will be reflected in the percentage pollen composition of the peat samples throughout the bog, though the absolute values for alder and oak pollen may for various reasons vary very widely. Thus the method of expressing the frequency of each tree pollen as a percentage of the whole can be trusted to reflect changes with time in the percentage composition of the woodlands. The *direction* of such changes will be more clearly shown than the *magnitude*. Nevertheless, when several genera are concerned changes in the relative abundance of the different genera afford extremely clear indication of climatic or edaphic changes, and it is the recognition and evaluation of such changes that constitute the pollen analytic method. That is to say, the method is not one dependent on the comparison of single samples from scattered sites, but rests on the comparison of the drifts evident in percentage composition throughout a number of vertical series of samples. Naturally in such comparisons the level in a series at which any particular tree pollen first appears also ranks as a horizon of great importance, since it may be taken as roughly indicative of the date of immigration of the tree. At the same time, since the pollen of the new species at first forms only a very small part of the total tree pollen, only extremely large or numerous pollen counts can be trusted to record the earliest date of immigration.

In the comparison of the time drifts of the pollen composition curves it is usual to find remarkably close general correspondence in the vertical series from any one locality or region. Thus diagrams *a* and *b* of Fig. 7 represent two separate series of analyses from the face of a peat bog exposed by coastal erosion at Skipsea on the east coast of

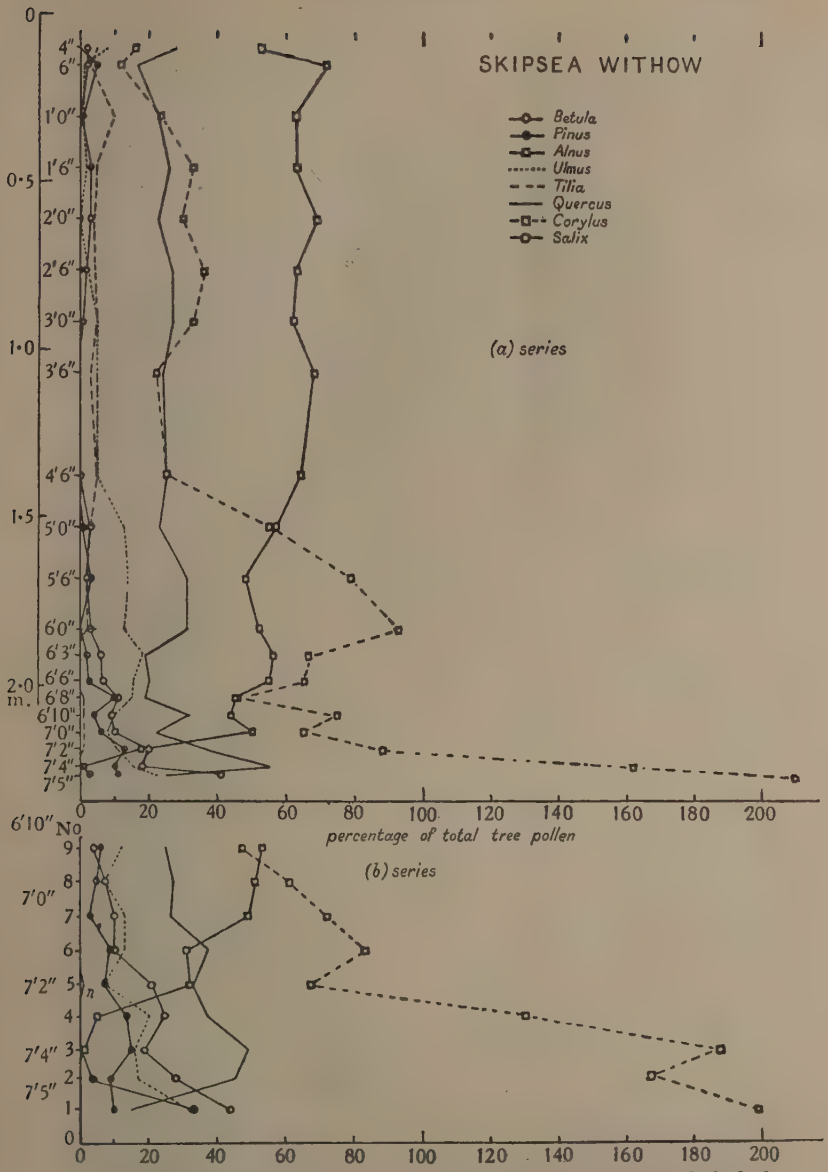


Fig. 7. Pollen analysis of a vertical series of peat samples taken through the bed of an ancient mere now being exposed by erosion on the Yorkshire coast at Skipsea. The upper diagram shows the complete sequence; the lower diagram is of the same site but is derived from a closer series of samples taken through the base of the peat bed some months after the first series. (Reproduced from *Antiquity* by kind permission of the authors.)

Yorkshire (13). Series *a* was collected and analysed with the results shown, and some months later series *b* was collected by a return visit to the original site. The later series was intended to give a more detailed picture of the basal few inches of the peat bed than that given by series *a*, and samples were therefore taken at much closer intervals though within a few inches laterally of series *a*. The correspondence between the two separately analysed series is extremely striking, nor could one mistake from direct inspection the levels corresponding in age in the two series. This is the condition generally found, although, where the comparison is between two series taken some distance apart, the vertical scales through the series may have to be considerably modified in order to get correspondence in the drift of the pollen curves. This is naturally a result of varying rates of peat formation, and though the difficulty it causes can be met by careful consideration of the pollen diagrams, yet it prevents any satisfactory treatment by direct statistical methods.

### POLLEN DOWNWASH THROUGH THE SOIL

It has been suggested (Malmström (23)) that downwash of pollen through the soil by descending water, especially along frost cracks, might be a fertile source of error in the pollen analysis method. It is likely to be of least importance in fine subaquatic inorganic deposits and most in open rapidly forming peat. Nevertheless Erdtman's investigations (5) seem to indicate that this source of error is not important even in *Sphagnum* peat areas. Erdtman investigated the pollen content of growing *Sphagnum* tussocks (Bulten) both in their surface layers and also at depths of about 0·5 m., corresponding to the surfaces of the hollows between the tussocks. The surface layers of these hollows (Schlenken), which are apparently not forming peat nor incorporating present-day pollen, were also investigated and were found to have a pollen content exactly comparable with that from the samples 0·5 m. below the surface of the growing tussocks. Such could hardly be the case if downwash of pollen were affecting the peat either of the tussocks or of the hollows.

It has also been pointed out by Erdtman (5) that the great conformity of the drifts in pollen diagrams of deposits formed at very different rates, very strongly opposes the possibility of pollen downwash. If it is to be assumed that pollen downwash has taken place in these deposits, it is necessary also to assume that in each case its effect has been proportional to the rate of formation of the deposit.

There is a good deal of similar evidence against the likelihood of such serious general errors, and Malmström himself later (24) showed, by direct experiment with *Lilium* pollen, that even in peat soils there is no substantial pollen downwash. Nevertheless, the possibility of such error must always be envisaged in interpretations of the presence of scattered pollen grains out of place in the general sequence of results of a regional analysis.

### Pollen spectrum as index to forest composition

It has been already indicated that differential pollen preservation must impair to some degree the accuracy with which the fossil pollen content reflects the composition of former woodland. The error so introduced is clearly indicated in the parallel analyses of wood, pollen and other tree remains shown in the following table.

*Remains of woody plants found associated with Bronze Age hut sites at Dullenried (Federsee). After Bertsch* (3).

| Tree | Wood from hut structure % | Pollen from culture layer % | Various remains (wood, leaves, pollen or fruit) |
|---|---|---|---|
| Populus } | 27 | 0 | + (tremula) |
| Salix } | | 0 | + |
| Corylus avellana | 23 | 10 | + |
| Alnus | 21 | 10 | + (glutinosa) |
| Fraxinus excelsior | 10 | 0 | + |
| Betula | 9 | 2 | + (verrucosa) |
| Fagus sylvatica | 3 | 44 | + |
| Acer pseudoplatanus | 3 | 0 | + |
| Malus (? communis) | 0·6 | 0 | 0 |
| Carpinus betulus | 0·6 | 0 | + |
| Taxus baccata | 0·2 | 0 | + |
| Quercus | 1·5 | 9 | + |
| Ulmus | 0 | 1 | + |
| Tilia cordata | 0 } | 2 | + |
| T. platyphyllos | 0 } | | + |
| Pinus | 0 | 7 | + |
| Picea excelsa | 0 | 11 | + |
| Abies | 0 | 4 | + |
| Sorbus aucuparia | 0 | 0 | + |
| Prunus spinosa | 0 | 0 | + |
| Frangula alnus | 0 | 0 | + |
| Sambucus nigra | 0 | 0 | + |

It is remarkable how far the wood percentages, calculated on several hundred samples, differ from the pollen percentages. Thus hazel, willow and aspen constitute over half the wood total. This is probably due in part to selective gathering for hut construction, a suggestion borne out by the wood analyses of the *piles* of lake dwellings on other sites where quite other trees, such as oak, ash and pine

predominate. From our present viewpoint it is, however, particularly interesting to note that the pollen analyses give no clue to the presence of *Populus*, *Salix*, *Fraxinus*, *Malus* and *Taxus*, which together constitute 41 per cent. of the wood samples.

Results of similar significance are given from several other sites by Bertsch, who also compares charcoal analyses from prehistoric hearths with the contemporaneous pollen analyses. In the latter case it might be expected that there would be less selective gathering of wood, and it does appear that closer correspondence exists here between the indications of the fossil wood and the fossil pollen. It is perhaps of interest to state that the Dullenried sites of the table given above occur at the beech maximum in the generalised pollen diagram of the Federsee region reproduced in Fig. 16 in Part II of this paper.

### (*a*) *Pollen production*

The effects of extreme susceptibility to decay extend, however, to a few relatively unimportant forest trees, and it is reasonable to expect, apart from this, that pollen analysis will afford an immediate key to former forest conditions. Nevertheless, many factors can be envisaged as likely to complicate this simple relationship, one of the most important being the different magnitudes of pollen production in various tree genera. Realisation of this has led numerous workers (Erdtman (5), Rudolph and Firbas (29, 30), Hesmer (15) and Aario (1)) to make direct comparisons between the pollen content of the surface layers of living bogs and the tree composition of adjoining forest areas. Hesmer has summarised their conclusions (which do not agree in detail) in the following series of diminishing pollen production:

Pinus > Corylus > Alnus > Betula > Carpinus > Abies > Picea > Fagus > Quercus > Tilia.

The dotted line represents approximately the point of best representation of forest composition by the pollen spectra. It is usually agreed that *Pinus* is always greatly over-represented [1] by its pollen and oak, and lime much under-represented. Whilst these extreme types are readily agreed to, the position of the intermediates on the scale is less well established. This is no doubt owing to the fact that the pollen amounts have to be expressed as percentages in which each component affects the others. Thus pollen spectra from a pine-beech neighbourhood will certainly show beech more under-represented than those from a fir-beech region, and beech would even be *over*-repre-

[1] But see p. 299.

sented in spectra from a beech-oak district. The only effective means of reaching finality in such a series would seem to be in testing it by contrasting each tree in the scale directly with that above and below it, and in ensuring the absence of other genera in appreciable amounts. Suitable regions for testing such pollen production, with the two given genera more or less equally abundant, would certainly often be difficult to find, and the results would be confused by the facts of variable pollen production at different altitudes, in different climates and on different soils, by the different sizes to which trees grow under these varied conditions and the variation in the age at which they commence to produce pollen. Such considerations make clear the very general character of the pollen-productivity series set out above.

A particularly interesting illustration of the results of comparing pollen deposition with contemporary forest composition is seen in the case published by Rudolph and Firbas in 1926 and illustrated by Fig. 8 given below. Superficial peat samples were collected from different altitudes in the Riesengebirge, so that seven samples were obtained between 740 and 950 m. in the forest belt with dominant spruce (*Picea*), abundant fir (*Abies*) and beech (*Fagus*), three samples between 1040 and 1220 m. in the pure spruce belt, six samples at about 1240 m. in the margin between the spruce and mountain pine (*Pinus montana*), three samples from mountain tops in the dwarf pine belt (*P. montana*), and various samples above the upper limits of the dwarf pines.

In samples from the lowest forest belt the spruce pollen is dominant. *Fagus*, though abundant as a tree, gives only 12 per cent. of the pollen, whereas the pine, quite absent from the woods, is represented by distant wind carriage to the extent of 20 per cent. The fir is even less well represented than the beech. Throughout the spruce belt spruce pollen is dominant, but at the margin of contact with the pine belt the pine pollen is by far the most abundant and remains so through the dwarf pine belt. On the treeless mountain tops, however, there is a striking increase in the pollen from the lower mountain zones. This is due to the weakening of local forest influences which now allow the effects of long distance wind transport to appear. Pollen such as that of oak, lime and elm must in these cases have travelled at least 10 km.

It would appear from these results that conclusions from the pollen spectra as to forest composition can only be of a very general character, but it should at the same time be observed how the

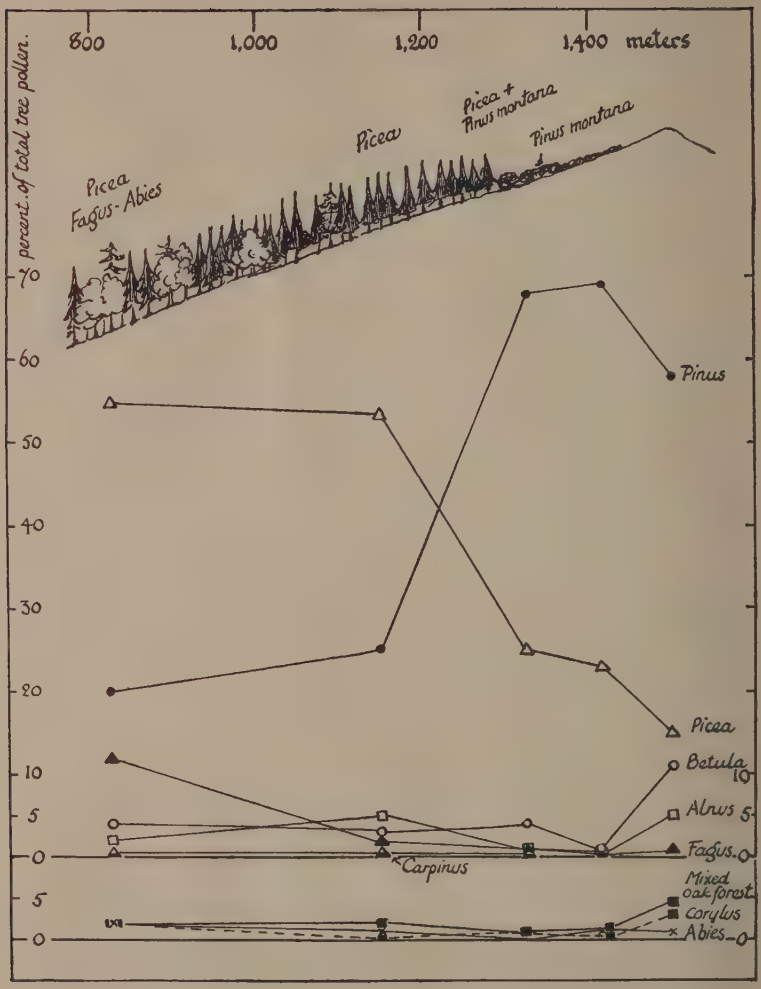


Fig. 8. Diagram to show the degree in which pollen samples from the surface of growing bogs reflect the composition of the surrounding woodland. The upper diagram shows the forest zonation on the Riesengebirge. Below are given the pollen analyses—each of the five results shown is the average of a number of samples taken at a given altitude. Though the beech, spruce and pine curves show their maxima in the proper sequence and roughly at the proper heights, yet the composition of the pollen samples differs very considerably from the forest composition (after Rudolph and Firbas).

maxima of the beech, spruce and pine in the pollen curves do reflect the actual vertical sequence of forest belts.

### (*b*) *Wind transport*

The illustration here quoted from Rudolph and Firbas draws attention to another source of error in deducing forest composition from pollen spectra, namely the variable transportability of pollen of the different tree genera.

Though exact data of the carriage of tree pollen are difficult to obtain there is considerable evidence that they can be transported for very great distances. The data under this head were collected by Hesselmann (16), who quotes the following instances:

Surfaces exposed on light ships in the Gulf of Bothnia at 30 and 50 km. from the coast trapped pollen of spruce, pine and birch. Water samples taken from 5 m. deep in the North Sea and almost 300 km. from the nearest land contained forty pollen grains of conifers per litre.

It has also been shown that plates exposed in aeroplanes at considerable altitudes will trap pollen. The local incidence of rust attack on crops has been shown by Canadian workers to be due to wind-blown spore infection from the south, and rust spores (some still viable) have been found as high as 17,000 ft. (31) In these investigations pollen grains were found abundantly at all heights up to about 10,000 ft., and Dillon-Weston reports similarly upon investigations in England (4).

Probably the most striking instance of long-distance wind carriage is, however, the case quoted by Malmström (24), who exposed plates to trap pollen at Degerö Stormyr and found spruce pollen caught there some weeks before the male cones of the spruce in the neighbourhood first opened. Malmström suggested that this pollen must have been transported from southern Sweden, a distance of 700–1000 km.

It has been very generally held that the winged conifer pollen travels further than that of the Angiosperm trees. Firbas (11) in a recent paper, however, produces evidence which discounts the assumption that the winged conifer pollen is particularly liable to over-representation by long-distance wind transport. He quotes direct measurements of the rate of fall of different tree pollens in still air, from which it appears that though the air sacs do have a large effect, it is offset by the large size of the conifer grains, so that the conifers show somewhat higher rates of sinking than some other species such

as alder. The known cases of very high values of conifer pollen in unwooded regions are attributed to other causes such as the great local preponderance of conifer woods in the region. Where there is no such disproportion in the source of wind-blown pollen Firbas fails to find evidence for increasing percentages of conifer pollen through differential sorting by winds blowing from wooded into woodless regions.

Though these arguments dispose of the possibility of using high conifer pollen percentages as evidence of former low density of tree cover, Firbas suggests that the ratio of non-tree pollen to tree pollen can be utilised as a very efficient guide to this condition, since only in very poorly wooded or unwooded regions does this ratio exceed one. The extremely local distribution of non-tree pollen, however, makes it undesirable to argue from single samples as to the tree poverty of a large area. It is quite clear that if there is differential pollen transport the pollen will be sorted out by winds blowing from the forest margin, and the distance of a deposit from the surrounding woods will be a factor influencing the composition of the pollen rain. Hesmer has proposed the following classification for the various possible components of the pollen rain on a basis of transport distance.

| | | |
|---|---|---|
| Bog tree component | ... | From trees growing on the bog[1] |
| Marginal ,, | ... | From trees on mineral soils round the bog |
| Regional ,, | ... | From the region up to 500 m. from the bog but excluding the margin |
| Distant ,, | ... | From 500 m. to 10 km. |
| Long distance component | ... | Over 10 km. |

It is evidently of great importance to determine which components are most strongly represented in any particular analysis; evidence of this may come not only from the pollen data themselves but also from macroscopic fossils, by the stratification of the moor and the knowledge of its development and topography. In most *hochmoor* peat deposits Hesmer considers the Regional component to be far the most strongly represented.

In this fact lies at once the strength and weakness of the pollen analysis method. It does give as a rule a *general* picture of the forest cover over a wide neighbourhood; not merely a parochial picture of the conditions, perhaps highly unusual, in some particular small area on or near the bog. That is to say, the conclusions from a pollen analysis series can be trusted as a rule to convey the general drift of forest

[1] "Bog" is used as a conventional translation of the German "Moor."

history over the whole countryside. The tree pollen, moreover, is produced so abundantly that if it is not destroyed traces at least may be expected of all the genera present.

On the other hand, this smoothing out of detail has its disadvantages, and where long-distance transport is possible over hundreds of miles the presence of scattered grains of any given species cannot be accepted as proof that the species is, or was, growing only in the neighbourhood; for this much less readily movable remains, trunks, branches, fruits and leaves must be identified, and the chances of finding such are often very slight, since many species are seldom to be found living sufficiently close to growing bogs for incorporation and preservation to have taken place.

It will, of course, be recognised that affecting problems of pollen dispersal by wind are such factors as the prevalent wind direction during the period of pollen liberation, and the effects upon wind currents of special topographic features. It is not necessary, however, to say more than that there is evidence for such factors having operated in certain special cases. The suggestion has also been made that the height of the pollen-producing "flowers" upon the tree must influence their dispersal and also the extent to which the grains cling together in groups of two or more. In view of the extreme heights at which pollen has been caught it seems probable that the first of these two factors may have a very slight influence indeed. Distribution of pollen in groups is particularly of importance in that single large groups may occur in a single peat sample, thus causing wide divergence from the normal composition; Erdtman has stressed this possibility, especially for *Alnus* and *Betula*, portions of the catkins of which are frequently found in peat.

### (*c*) *Flotation and water transport*

It is probable that rivers bring into lake- and flood-plain deposits considerable amounts of pollen, especially from trees such as *alder*, which grow abundantly on the river banks. Auer (see Hesmer (15)) has also shown that pine pollen can be transported in this way. The conifer pollen appears to differ considerably from Angiosperm pollen in its powers of flotation, and where present in considerable amounts forms the conspicuous "Seeblüten" of continental lakes. Winds and currents carrying the surface water to the shore would tend to raise the conifer pollen percentage in the shore deposits compared with those forming in the centre of the lake. Certain observations by Malmström and Lundquist (22) appear to support this view, but as is

pointed out by Wasmund(36) and Hesmer the complexity of the factors involved in fossilisation forbids any prediction of over- or under-representation for conifer pollen in particular parts of the lake deposits.

### (*d*) *Time of flowering*

The pronounced maximum for hazel pollen shown at the base of the Skipsea diagrams (Fig. 7) is an extremely frequent phenomenon in British and continental peat deposits and has led to the suggestion that at certain times in the post-Glacial period, especially in the Boreal period, the hazel formed real forests dominating extensive areas of Europe. This extremely interesting possibility has been reviewed by Erdtman(8), who published the accompanying map (Fig. 9) to illustrate the periods of post-Glacial time at which hazel pollen maxima have been found in the various parts of Europe where pollen analyses have been made.

It will be seen that the boreal hazel maxima especially characterise central and western Europe, the British Isles have hazel maxima at all parts of the post-Glacial time scale, there are no boreal maxima in the zone of the Vistula-Dniestr, which stretches from the Baltic to the Black Sea, and east of this the pollen maxima are post-Boreal. In the Baltic the maxima occur mostly in the Atlantic period.

Erdtman draws attention to the extremely early date of flowering of the hazel, and suggests that this becomes of great importance with regard to pollen incorporation in peat-forming bogs. Bogs which are still frozen will, he suggests, be incapable of retaining the pollen blown on to them, it will not be washed down to the wet anaerobic layers of the bog and will decay, leaving no trace in the pollen content of the bog of this rain of hazel pollen. Such, he considers, may have been the explanation of the absence of the hazel maxima from the Vistula-Dniestr zone, where, especially in the boreal period, continental climate may be supposed to have prevailed with accompanying late springs and long-frozen bogs. Similarly the pronounced hazel maxima at all parts of the post-Glacial period (after the subarctic) in the British Isles may be linked with the early springs which accompany a maritime climate. The climatic optimum Erdtman supposes also to have been characterised, in all but eastern Europe, by early springs, and since this optimum occurred in the Boreal in central Europe and in the Atlantic in southern Sweden, this, again, coincides

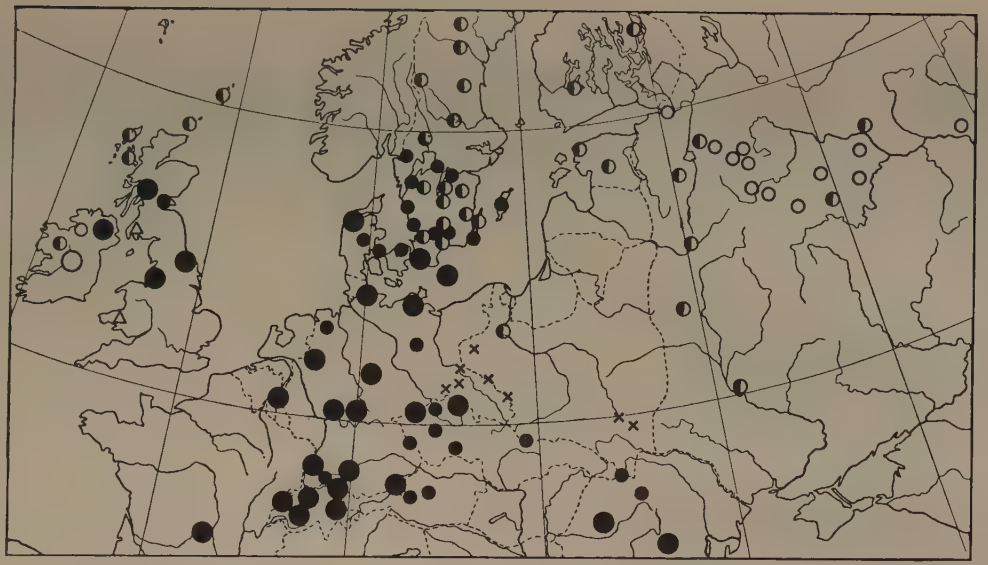

Fig. 9. Map showing the different parts of the post-Glacial period at which hazel maxima have occurred; after Erdtman(8). (By courtesy of the *Journal of Ecology*.)

- • Maximum in the boreal period (pollen frequency < 100 per cent.)
- ● ,, ,, ,, ( ,, ,, > ,, ,, )
- ◐ ,, ,, atlantic period ( ,, ,, < < ,, ,, )
- ○ ,, ,, sub-boreal period ( ,, ,, < < ,, ,, )
- ○ ,, ,, ,, ,, ( ,, ,, > ,, ,, )
- △ ,, ,, sub-atlantic period
- × No distinct maximum: pollen frequency low.

with the suggestion that the hazel maximum may be a reflection of early springs and active bog growth.

It would clearly follow from the arguments thus advanced that on the one hand a hazel pollen maximum is not necessarily a criterion of a corresponding fluctuation in the importance of hazel *forest*, and, on the other, that hazel may be present abundantly without leaving indications of this in the hazel pollen record of the local bogs.

It is evident that the pollen analysis data must not, in this case at least, be interpreted without careful consideration of other lines of evidence, particularly those relating to climate. The hazel pollen maximum of central Europe is, however, valuable as a chronological index whether it does or does not accurately reflect a phase of forest history, because in either case it seems the response to a widespread climatic change.

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# COMMENTS ON RECENT STATEMENTS REGARDING THE NATURE AND ORIGIN OF THE ANGIOSPERMIC STIGMA

By J. McLEAN THOMPSON

"A man has a scientific judgment only on such objects as he is acquainted with through his own researches; how many may there be who have ever once attempted to form an independent opinion of the nature of the organs of propagation of plants by the investigation of their development on only one single plant?"

J. M. Schleiden, *Principles of Scientific Botany*, p. 374 (1849).

In a recent article entitled "The Nature and Origin of the Stigma"[1] Dr H. H. Thomas invites criticism of his view on the history of flowering, and challenges those who hold opinions other than his own to reconsider and state the foundations of their beliefs.

Response to this invitation might, meantime, be withheld but for statements by Dr Thomas which call for correction.

It is first necessary to examine the approach of Dr Thomas to angiospermic flowering as revealed in his earlier writings, and in particular to comment upon his statement to the Linnean Society of London on "The Old Morphology and the New." Following a communication upon "The Early Evolution of the Angiosperms" in which it is affirmed that "there seems to be little prospect of solving the problems of the origin of the Angiosperms from the comparative study of modern forms," and that "we must search through the remains of fossil floras for clues to possible solutions," Dr Thomas turns to the Jurassic flora.

He adduces the Caytoniales as having "evolved angiospermy in Lower Jurassic times, apparently by the closing of a cupule-like envelope around the ovules and the formation of a stigma." Some similarity to both Pteridosperms and Angiosperms is indicated, and on this alone comparisons are offered between the cupule-like envelopes of the seeds of *Caytonia* and the modern carpels of Ranunculaceous and Rosaceous species. As to the validity of such comparisons there is no evidence; nor is the implication that the problems of angiospermy are insoluble on the testimony of Angiosperms themselves justified.

[1] *The New Phytologist*, **33**, No. 3, June, 1934. *Note.* The reader is referred to the bibliography given in Dr Thomas's paper.

A second step in approach to the problems of angiospermic flowering is revealed in "The Old Morphology and the New." And here, following pronouncements on subjective and objective reasoning, and the statement that "most scientists aim at an objective approach to their objects of study," it is shown that Dr Thomas has become aware of new conceptions of flowering already expressed by others from their direct study of living Angiosperms. He constitutes himself the voice for such conceptions and proceeds as follows. "In groups like the Ferns, Lycopods, and certain Gymnosperms...both ontogenetic study and vascular anatomy have been shown to have very considerable significance, and there is every reason to suppose that the same is the case in the Angiosperms."

If, with this supposition before him, Dr Thomas had joined with others in the study of angiospermic ontogeny, he would not have relapsed, as he did with startling rapidity, into what he had deplored as subjective reasoning in seeking a more intelligent view of floral evolution. For his statement proceeds, "I have already suggested the derivation of the carpels of the *Caltha* type from the Pteridosperms *viâ* a structure of the *Caytonia* type." The cupule-like envelopes of the seeds of *Caytonia* become, without further evidence, cupules, reduction of the *Antholithus Arberi* type of the Caytoniales to a single stalked anther is conceived, and the type of stamen possessed by *Ranunculus* is reached.

A more subjective approach to modern angiospermy may not readily be imagined, and while "neither delighting in wild speculations nor enjoying an outburst of iconoclasm," Dr Thomas leaves his audience to form their own conclusions as to the bearing of the Caytoniales on the numerous problems of modern angiospermy.

In commenting at this stage on the approach to modern angiospermy herein revealed, the present writer, from personal contact with floral development and adult structure, remarked that "no greater dis-service could be done at the present stage to our appreciation of the problems of the living Angiosperms than to accept readily the reading of their history in terms of ancient floras. The true service of palaeontology to the history of the angiospermic flower will begin with the provision of undoubted Angiosperms, with their record of reproduction, from the rocks themselves."

A third step has now been taken by Dr Thomas in "The Nature and Origin of the Stigma." A subjective outlook is again deplored, direct observations on angiospermic flowering are swept aside, postulates (which, it is presumed, are bases of argument laid down as well

known or too plain to require proof) are provided, and the view that the angiospermic carpel has arisen from a *pair* of concrescent cupules is now advanced. The margins of the inner surfaces of the cupules provide the modern stigma, placentae are fertile branch tips originally surrounded by a cupule, and it is stated that this view agrees with what we know of the ontogeny of carpels.

On these and other points comment may now be offered.

And firstly, following a statement that some, if not all, of the old canons of floral morphology may eventually prove to be well founded, but if so, they must be based on arguments differing radically from those which have hitherto been advanced; that, "at the outset we must do our utmost to avoid a subjective outlook," and that the relationships of the parts of different flowers must be viewed from the standpoint of their possible evolutionary origin, and not merely as parts of a mental process, the theme runs as follows: "It may be argued that there is little difference in practice between a subjective and an objective approach, but this is not true. The subjective treatment of morphology regards form as an intuitively apprehended concept, and consequently considerations of physiology and phylogeny have no practical significance. Crudely stated, the *Caltha* carpel is regarded as a folded leaf, because it looks like one. The objective attitude takes the material of which the plant is built, and considers it in relation to the processes going on within it and to its environment."

It may here be remarked, with equal crudity of expression, that the envelope of the seed of *Caytonia* may be, and is regarded by Dr Thomas as a cupule, because to him it may look like one. Consideration of processes within and without the cupule-like envelope is for ever debarred.

So much for the objective approach as exploited by Dr Thomas in matters of floral morphology, and for argument "differing radically" from that which he has claimed to expose.

Turning now to his statement on the foundations of a system of morphology, he affirms that "all modern systems of taxonomy rest on one fundamental proposition, viz. that the different parts of the flower represent modified foliage leaves." Refutation of this statement is scarcely necessary, but is here made in view of what is declared to be its corollary, namely "that all the floral parts are referable to an original and continuous spiral sequence even though they show no trace of a regular sequence in their ontogeny." As to how a spiral sequence of floral parts must constitute a corollary from any belief

as to the origin of floral organs is not self-evident. It may further be remarked, for it is in accordance with the truth, that spiral succession of parts is widespread in modern angiospermic flowering; it may be observed by anyone engaged in ontogenetic study and is no assumption such as Dr Thomas has seen fit to suggest.

But his statement proceeds as follows. "The consequences of such an assumption are illustrated in a recent contribution to the discussion of carpel morphology. In this paper Prof. McLean Thompson assumes a spiral succession of the structures we are accustomed to call the placentae of the carpels.... Mere numbering of primordia in diagrams affords a poor foundation for a far-reaching theory."

Correction of this statement is necessary, for at no point in his "Theory of Scitaminean Flowering" has the present writer assumed or even suggested a spiral succession "of the structures we are accustomed to call the placentae of the carpels." Nor has he advanced a mere numbering of primordia in diagrams as a foundation for any theory. He had followed the entire ontogeny of each Scitaminean species described, has observed the spiral succession in each species from bract to final stylar component, has traced with care the rise to prominence of the placentae beneath the stylar components, and has made it abundantly clear that the placentae *do not* arise in spiral succession. He has numbered in diagrams the primordia of floral parts, species by species, in accordance with the observed succession alone.

Further obvious deductions advanced by Dr Thomas from his statement on modern systems of taxonomy are that the follicle is the most primitive carpel type, and that no carpel or group of carpels can be regarded as primitively terminating the apex of the flower. These deductions are dispatched as based on unsubstantial grounds.

It will be shown later that neither the interpretation of the follicle nor the terminal position of a carpel or group of carpels depends on a belief in the foliar origin of floral parts.

Having satisfied himself of the insecurity of an alleged fundamental proposition and its alleged corollaries, Dr Thomas states that "it thus becomes necessary either to produce fresh fundamental evidence or to set aside these theories, with all that was derived from them, as assumptions which cannot be proved, and to endeavour to build up a new system."

The re-entry of the Caytoniales into the field of discussion is clearly indicated, and is heralded by postulates for any system of morphology. In brief, the primary postulates offered are as follows: (1) that vascular plants have evolved from simple ancestors, (2) that

the earliest differentiation of ancestral forms was that of reproductive from somatic cells, and (3) that structural or physiological mutations advantageous to the race tend to be selected.

The immediate bearing of these profundities on the matter in hand is obscure. They, however, lead Dr Thomas to suspect the present writer of belittling hereditary characters because he has studied time-factors in growth, the form, state and stature of floral receptacles, and the number, form, stature and distribution of floral organs and what occurs within them, and accepts each form of flower as an individual expression of the problem of flowering. It may rightly be remarked that if, in recording meristic variations, the present writer has assailed inheritance, something should be done about it, and that neither the existence of seed-bearing plants with cupule-like appendages in Jurassic times, at present known, nor arguments based thereon may serve to mask the problems of modern flowering which for long have been the special study of the present writer.

The final postulate is that all known vascular plants are descendants from the same or similar ancestral stocks, have essentially similar physical constitution, and that in consequence a uniform system of morphological concepts should be applicable to all. Ferns and Angiosperms are thus admitted to comparable ancestral history and, under the mantle of the former, the Caytoniales are at last ready to enter the arena to handle the problems of angiospermy, though sharing with their guardian defects of subjectiveness.

Their qualifications for the interpretation of modern angiospermy are not, however, presented until a further essential for a morphological system is explained, namely that it must be applicable to all facts of structure and development. The gamut of carpel ontogeny, mature form and structure, carpellary vasculation, styles and stigmas, transmitting tissue for pollen tubes, abnormalities, the results of experimentation, ovule form and placentation, and fossil records are admitted as evidence in the reading of carpellary history. The modern androecium and perianth are, however, excluded from enquiry, despite an earlier statement that "we must consider the floral organs as interdependent parts of a whole living plant," and the fact that they antedate the carpels in ontogeny on a common receptacle. How fatal is this omission to the cause of the Caytoniales will later be indicated.

At this point Dr Thomas comes to grips with the problems of angiospermy and firstly with the questions as to how, when and why did the stigma arise.

He disposes of the conception of an ancient glandular tip of an expanded fertile leaf as raising questions which cannot now be answered; he rejects the inrolling of leaf margins before the evolution of pollination through the medium of a stigma as involving a mutation unfavourable to the race, and considers it unlikely that the appearance of the stigma and the inrolling of the leaf would occur simultaneously in a single mutation.

Having already stated that all known facts of structure and development are significant, he now turns to facts of development of stigmatic styles as recorded by the writer in his "Theory of Scitaminean Flowering." It is there shown that the Scitaminean styles are potential stamens, and that no valid opinion on the nature of the inferior ovary in this affinity can be reached without consideration of the flower as a whole. In the absence of detailed descriptions of the stigmatic surfaces of the styles Dr Thomas states that the writer's view of androecial origin for the styles "appears to have little objective foundation," and "cannot now be usefully discussed," despite the fact that it offers the first "objective" statement on stylar origin.

Dr Thomas's statement of the interpretation offered for the Scitaminean flower is next in error, for he quotes the writer as regarding "the primitive flower" as possessing only vegetative emergences, stamens, and stalked ovules borne on the crater-like receptacle.

At no point has the writer offered a view on the organisation of "the primitive flower," nor has he conceived as primitive a crater-like receptacle as above described. He has, however, suggested from intimate study of Scitaminean flowering that in the immediate ancestry of the *Scitamineae* the flower was as depicted, and to this suggestion he is inclined to adhere.

Dr Thomas now turns to the nature of the stigma of living plants. He quotes from the observations of Capus, Guéguen and Juel on the presence of glandulation in the ovaries of certain living Angiosperms and on the path of pollen tubes, and feels justified, without any ontogenetic knowledge of the styles or ovaries selected, in regarding the stigmatic surface as a specialised part of the inner wall of the ovary commencing basally and extending upwards. He declines to accept the view that stigmatism may declare itself first at the apex of a style, although it is manifest in many affinities that stigmatism is thus accomplished. The truth may partly be covered by the statement that there is no such organ as a stigma, that stigmatism is of varied locus, extent, inception and duration, that it may occur

externally to the ovary, and that it expresses a state of tissue in some way associated with limitation of growth.

There is no evidence for the conclusions of Dr Thomas that "the stigma originated on the ventral side of the carpel, that subsequent development of the style, or of the ovary wall has gradually carried it upwards, and that it is a structure appearing to be composed of two adjacent lobes or parts, the contiguous facies of which are lined by glandular hairs and continuous with transmitting strands below."

The discussion on the nature and origin of the stigma is not, however, completed, and the Caytoniales cannot play their part without distraction until the carpels of *Drimys Winteri*, *Magnolia*, and others have borne testimony to the dual nature of the stigma: for it is now apparent that since each cupule-like envelope of the Caytoniales is to provide a stigma and that fusion of such envelopes in pairs is to be assumed in the making of a carpel, the stigma of this fusion-product must be dual. Dr Thomas's statement on *Drimys* may suffice to illustrate the approach. An advanced carpel is figured, its undivided and U-shaped terminal stigma is described as a double lateral structure, and the fact that glandulation is not restricted to a clearly circumscribed area, but extends to the adjacent placentae is held in some way to furnish support for his contentions.

A suggested origin of the stigma is now reached, and, on the matters analysed above, and on them alone, it is stated that "fortunately we are now able to outline a possible history of the evolution of the carpel which would co-ordinate the varied facts of structure and combine them with what we know of fossil seed plants. One may call it a history rather than a phantasy because it commences with a group of plants which actually existed, and is based on the comparison of the plants of successive ages."

The Caytoniales are the group of plants referred to. Their qualifications as interpreters of modern Angiosperms still remain—that they possess cupule-like envelopes for their seeds, dissociated in any strict sense from microspore-bearing organs, and that their affinity is unknown.

The final phase in Dr Thomas's statement is now reached. It is offered as a legitimate assumption that cupule-like bodies have fused in pairs, so that we should have a structure "which is in all essentials an angiospermous carpel." The original ovule-bearing stalks become adjacent placentae, the recurved papillate tips of the fused cupule-like envelopes form a pair of lateral stigmatic surfaces, and the whole ovuliferous structure is imagined reduced, so that the equivalent of a

modern gynoecium may be provided. In so doing Dr Thomas has thrown his principles of objective reasoning to the winds and draws his study of modern angiospermy to a conclusion satisfactory to himself. Such minor difficulties as the possible re-entry of the modern androecium and perianth (which, together with the major portion of his essentials for a morphological system, has been forgotten in his discussion) remain for later disposal.

In reflecting on the approach to angiospermy herein revealed the writer would point a moral from his initial quotation. With others he will welcome the testimony of authentic Angiosperms from the rocks, and until this testimony is provided the Caytoniales, as at present known, will remain—the Caytoniales.

And lastly, Dr Thomas has requested a statement on the foundations of the beliefs of others. In so far as the writer is concerned a statement is gladly offered. He believes that by the study of living Angiosperms the essential problems of angiospermy may be exposed. The foundation of this belief is observation from the floras of many lands. By such observation he has reached tentative views on some of the salient problems of modern angiospermy, and they are as follows:

1. That in all species which he has examined the floral receptacle is potentially heterosporous.

2. That microsporangia and megasporangia alone are essential products of the receptacle.

3. That anthers and nucelli are these essential products.

4. That curtailment of the receptacle is widespread and progressive both ontogenetically and racially.

5. That, as in leaf formation, this curtailment leads to emergence of the receptacle.

6. That emergence is compensation for failure of apical growth.

7. That by such emergence anthers and nucelli are raised from the receptacle, and stamen filaments and funicles are provided.

8. That with limitation of growth of nucelli integuments likewise express diverted growth beneath the nucelli.

9. That when, in ontogeny, the limits of apical growth of the receptacle are imminent, while microsporangium formation is slowly progressive, successive emergences are formed. Each emergence temporarily satisfies the needs of growth, the microsporangium is "broken" into minor tracts, and numerous stamens result.

10. That when limitation of apical growth is more rapid fragmentation of the microsporangium is less marked. Emergences are

fewer, individually they are relatively massive, and the resultant stamens are few and relatively large.

11. That sterilisation of the upper zones of microsporangium commonly occurs.

12. That emergence of the receptacle by toral growth may be added to discrete emergence at any point in ontogeny at which the latter no longer fulfils the requirements of growth.

13. That such toral growth determines the final form of the flower according to its incidence and persistency.

14. That when stamen formation is still in progress and toral growth is added, both perianth and androecium may be raised on the torus so that some members of the androecium are on or near the toral rim.

15. That these upper stamens are commonly sterilised, constitute the styles, and later become glandular.

16. That the basin formed by the torus is purely receptacular, is throughout potentially megasporangial, and that as it is extended it bears emergent nucelli varying in both number and size, as is the case with the anthers and their filaments.

17. That the original diameter of the basin limits expansion, that in-growth occurs in varied degree beneath the styles, and that placentae are thus created.

18. That placentae are the tracts of the basin best nourished and on which ovulation is most readily maintained in what is now known as inferior ovary.

On this view there are no carpels involved in the organisation of an inferior ovary, their existence need not be considered a necessary prelude to ovulation or as intervening between microsporangia and megasporangia, and there is no sinking of bodies of any form whatsoever into the receptacle. The state of angiospermy is fulfilled, and limitation of apical growth accompanied by toral growth dominates the moulding of the flower. There is no call to consider this form of angiospermy inexplicable without reference to ancient fossils nor to stretch their scanty evidence into a phantasy and to call the latter a history. The writer has already furnished some short chapters in the events from a single living affinity, and will extend his statement to ovulation at an early date[1].

It is, however, apparent that until the facts of gynoecial ontogeny for some family with superior ovaries are made known many will

[1] Publications of The Hartley Botanical Laboratories, No. 12, *On Placentation and Ovulation*. A preliminary statement based on ontogenetic study.

rightly hesitate to share the writer's views. He is therefore providing facts of observation on the Ranunculaceae supporting the view that the early course of ontogeny is as above presented under headings 1 to 13, that in the formation of follicles sterilisation of stamens is involved; that by toral growth from beneath the arrested apex of the receptacle the sterilised emergences are variously united; that as the torus persists in growth it supplies the receptacular surface on which ovulation is expressed as in the inferior ovary; and that the distinction between superior and inferior ovary is merely one of description.

The matter is considered likewise in the formation of achenes, and again carpels as historic documents in the story of angiospermy are considered unnecessary.

The writer confesses to a return to a view of flowering not far removed from that of Schleiden of a hundred years ago, though it may be expressed in different terms.

In issuing these pages in response to what is considered improper criticism and approach to a complex problem he has been forced to anticipate reasoned statements on observations made, and believes that men of science will await with patience the story of the living Angiosperms.

# SUBSTITUTION STAINING WITH FREE DYE ACIDS AND DYE BASES

By R. C. McLEAN

THE acid dyes eosin and erythrosin in their usual states are sodium salts of dibromodichlorofluorescein and tetraiodofluorescein respectively. They are soluble in water but sparingly or not soluble in most organic solvents. The corresponding free acids are, however, soluble in ether or xylol and can thus be separated from an aqueous solution of the dyes.

This simple piece of dye chemistry has, however, an importance in microtechnique which does not appear to have found its way into the literature of histological practice.

*Procedure.* Make a 1 per cent. aqueous solution of either eosin or erythrosin. For every gram of erythrosin in solution add 2·5 c.c. of a 10 per cent. solution of hydrochloric acid of sp. gr. 1·16. For every gram of eosin add 3·5 c.c. of the same acid. Precipitation is immediate. The simplest procedure is then to allow the deposit to settle for 24 hours, pipette off the clear liquid (the slight excess of acid assists sedimentation), add pure xylol to the residue at the rate of about 200 c.c. for each gram of dye used and mix by gentle shaking or by stirring with a glass rod. Avoid strong shaking in the case of erythrosin, or a viscous emulsion may form. Pour into a separating funnel and continue gentle agitation as long as any solution is taking place, then allow to settle and run off the xylol extract, which will be practically colourless.

The dye acids are not highly soluble, so that if a stronger solution is desired the original precipitate should be filtered on to asbestos (not paper) in a Gooch crucible, air dried and finally shaken up with dry xylol in a mechanical shaker.

From the colourless solution the colour is again produced by the addition of alkalis or by an electronegative adsorbent such as cellulose, with which an ionic exchange can be effected. This may be seen by dipping a piece of filter paper into it.

Sections of plant tissue are first stained with methyl violet or methylene blue, washed as usual in methylated spirit and absolute alcohol to remove the stain from the cellulose walls, then transferred to xylol. From this they are placed in xylol eosin, when the red

colour develops immediately. Prolonged staining is of no advantage, the sections should be at once transferred to xylol balsam or some other resin, as permanent mounts.

Counterstaining is thus made a perfectly mechanical procedure, coming at the most convenient point of the operations, immediately before mounting, and all difficulties of differentiation are avoided.

Eosin gives a more orange tint than erythrosin, but otherwise their effects are the same. The colour formation is also a test reaction for cellulose in the wall, and it is interesting to observe that, while lignified tissue as a whole is unstained, the protoxylem vessels and those in process of formation at the inner cambial boundary show a half-red tint, while metaxylem and sclerenchyma are entirely colourless, unless counterstained. Cork and pith cells are also uncoloured, but in the latter tissue the intercellular trigones are strongly stained. Cytoplasm and plastids are lightly coloured or not at all, but nuclei are stained.

The free base of methylene blue (Nile blue is better) may be used in a similar way. Add 2·5 c.c. of 10 per cent. sodium hydroxide for each gram of dye in solution. Precipitation takes several hours. The xylol solution of the base has a slight purplish red tinge, but sections of plant tissues placed in it have their lignified membranes immediately stained bright blue.

It is not possible to combine the xylol eosin with this stain in one solution, but they may be used successively.

Place the sections first for a few moments in methylene blue xylol and then transfer directly to eosin xylol, when a perfect double stain is automatically achieved.

The process was originally suggested, though fallaciously, as a test for alkali in tissues. It avoids so simply the difficulties and uncertainties of other staining processes that it deserves further study.

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# REVIEWS

*Researches on Fungi.* Vol. v. By A. H. R. Buller: London: Longmans, Green and Co., 1933. 9·4 in. × 6·2 in. Pp. xiii + 416 with 174 figs. 25*s.* net.

Prof. Buller's *Researches* need no introduction. They rank among the foremost in mycology. Indeed, with the publication of each successive volume, we have learnt to expect issues of greater import and finer execution, and in these latest we are in no wise disappointed. This fifth volume proceeds from general topics of the mycelium by many diversions to the elucidation of one of the most specialised mechanisms of the Gasteromycetes, and for its discoveries, its conclusions and its clear and ingenious exposition we consider it to be the best. It is modelled on its predecessors; a generous summary and a wealth of illustration supply the essence, and one is guided through the details of an explicit text by a copious index.

The first part of the book, which continues vol. IV, deals with the subjects of hyphal fusions and protoplasmic streaming and will appeal particularly to the physiologist because it adds so much to our knowledge of the construction of the mycelium and of the interchange of materials within the hyphae. The various ways of fusing are described to the effect that all are "end-to-end" fusions representing "action at a distance" of one hypha on another. And in disproving Burgeff's explanation of this phenomenon (for the clear presentation of which we are most indebted) Buller points out that so ordinary an occurrence leads on consideration to a problem of the first magnitude, at present insoluble and demanding, perhaps, the concept of vital radiation. Experiments in hybridisation, by observation of hyphal fusions, give a check on the work of the systematist and support his conclusions, especially in the case of the Hymenomycetes, where species-making has been deemed a trivial affair. This should be a rich field for research.

Protoplasmic streaming in hyphae (at rates up to 55 $\mu$ per sec.) occurs in many fungi, though its significance can yet hardly be appreciated. "In a mycelium of *Pyronema confluens* protoplasm was seen streaming through 161 successive cells....The length of the stream exceeded 1·6 cm." There must be passage-ways through the septa, and Buller now demonstrates beyond dispute that the septa of all Eumycetes are persistently perforate in the centre, unlike those of Phycomycetes which are imperforate when fully developed and serve not for the transmission of protoplasm but for the retention of it. We are presented with the novel picture of the mycelium of a hymenomycete, diploidised perhaps by means of the septa and further compounded by fusions with other mycelia, through the hyphae of all of which, independently of their origin, protoplasm is propelled to a common end by the motive power of vacuolation. The fruit-body is inflated by this same force, which drives also the protoplasmic reserves from the mycelium up to the growing-points and ultimately into the extremities to be discharged in the spores.

There are important digressions too on clamps and septa in general, on "Woronin bodies," on rates of nuclear division and apical growth, on the effect of wounding hyphae and on regeneration. But few points we would contest. The toughness of the fruit body of *Marasmius oreades* (p. 2) is surely due not to the numerous hyphal fusions but to the slight thickening of the hyphal walls, the interweaving of the hyphae and their relatively slight inflation. Further investigation is needed to show how general hyphal fusions may be in fruit bodies, especially among polypores and thelephores. A closer analysis of the mycelium of *Pleurage curvicolla*, for instance, might have revealed a structural

unit corresponding with the physiological, e.g. a peg from each cell. On p. 66, line 3, "1900" is surely a mistake. The biological advantages attributed to the construction of septa and clamps are clearly effects, not causes, and we are still left in the dark concerning their origin. We gladly read that clamps are only analogous with ascogenous hooks, the supposed homology having always appeared artificial. But we should like to know the exact difference between vegetative (nutritive) fusions and sexual fusions.

The second part is in three chapters, each devoted to particular fungi. The first is a review of the Sporobolomycetes with a special account of *S. roseus*. Buller concludes that they are basidiomycetes. The spores have precisely the shape and mode of discharge of true basidiospores, and these properties, the author maintains, are the criteria for the homology of basidiospores, inasmuch as they are found in no other kind of spore: and, in view of the comparative evidence adduced from the smut basidium in the succeeding chapter, there is but the alternative to create a new phylum and beg the question. The author independently corroborates Guillermond's observations that the cells and spores are uninucleate, no binucleate phase having been discovered: and such anomalies as successive spore-production and branched sterigmata are readily accounted for. Those who consider fungi to be the subaerial expression of marine filamentous phytobenthon will welcome this discovery of a family of basidiomycetous yeasts; their theory postulates that yeasts shall be the last term in reduction of any fungus phylum. But is it proven that the Sporobolomycetes are not the basidiospores of smuts? Fig. 90 displays the futile simplicity of these little organisms which, expending all their energy in absorbing food, blindly get rid of it by an elaborate mechanism, in delicate spores, after a few hours and within the compass of a few microns. Can they be primitive?

The second chapter will probably be most widely read. It is the best account we have of the common class-fungus *Tilletia tritici*: accuracy in observation is combined with fullness and excellence of illustration, so that there seems nothing to be desired. According to his conception of the basidiospore, the author interprets anew the basidial apparatus of the smuts and introduces a revised terminology, aptly supported by a limited but effective comparison of the basidial apparatus in three other genera. It is greatly to be preferred to the Brefeldian terminology, current in text-books, although there is the regrettable anomaly—that "secondary sterigmata" should produce "primary basidiospores": such a term as "prosterigmata" might have been preferred for the "primary sterigmata." Characteristic of the thoroughness of Buller's methods are the descriptions of six variations in the development of the basidium and basidiospores, one only of which is really normal and that is here described for the first time. The septation of the promycelium is described in detail and many facts are gathered of the nuclear behaviour in smuts, of the water-absorbing power of the mycelium of *T. tritici*, of the close relation between germination of the basidiospores and drying, and of the chlamydospores and "ventilation," and of the negative hydrotropism of the promycelium, which is an astonishing variety of water-requirements in an endophytic fungus. Although *T. tritici* is highly specialised and reduced, it shoots away its spores with greater violence than any other basidiomycete. There appears a slip on p. 33 (three lines from the foot of the text): "secondary" should read "primary."

The final chapter is a history and description of the gasteromycetous genus, Sphaerobolus. The researches of Leva Walker are corroborated and extended, and we have now a thorough knowledge of the structure, working and kinetics of this marvel of hyphal engineering (though it still remains to be discovered exactly how it is developed from interweaving hyphae). The fruit-body is a miniature mortar with "the largest known fungus projectile" and it is "the largest, most powerful and the loudest of all fungus guns": little wonder it attracted the author's curiosity, especially when it hit the ceiling in his office. After describing minutely the structure, Buller shows how practically every feature of the fruit-body, macroscopic and microscopic, is concerned in the

perfection of the mechanism. The motive force is the rapid eversion of the inner peridial membrane (in 1/1500th sec.) and, though paralleled by the Geasters, in power and effect it is unique: it is, nevertheless, a hydrostatic mechanism as in all methods of violent discharge in fungi. *S. stellatus*, which has been known to science for 200 years, is reckoned a common species, yet Buller is the first to have shown that it is a coprophilous fungus of the most advanced kind, comparable with Dasyobolus and Pilobolus. The chapter closes, as one would never have foreseen, with an instinctive comparison between the two main types of coprophilous fungi. And, as befalls all upon which his wand alights in his love of mycology, *S. stellatus* becomes the best known of all gasteromycetes. We suspect the family of some phalloid origin, chiefly on account of the form of the basidia and spores, the gelatinisation of the peridium, and the glebal cavities found in *S. iowensis*. On p. 321 (one line from the foot of the text) for "osmotic pressure" it seems we should read "turgor pressure."

It is clear that these *Researches* are indispensable to the mycologist, be he physiologist, morphologist or systematist. And if they are a little laboured and in parts overwrought so as to be void of misunderstanding, there is a finality about them which we would have at any cost.

E. J. H. CORNER.

*Introduction to Cytology*. By L. W. SHARP. 3rd edition. McGraw Hill Co., 1934. 9 in. × 5½ in. Pp. 567 + xiv. 230 figs. in text. 30*s*.

The earlier editions of Sharp's *Introduction to Cytology* have been widely used by students in the universities of the United States and this country. They provided in a limited space everything that was deemed to be academically essential in the subject. They were compiled by a respected teacher. Their point of view was morphological and static, and could readily be assimilated by the student accustomed to other morphological and static branches of biology. The new edition, in spite of the difficulties created by recent innovations in the subject, preserves this point of view unchanged and its academic adaptation is perhaps even more skilfully carried out. It is again cautious, careful and complete. But the text is shortened and is now straightforward and unencumbered for the elementary student. The bibliography is lengthened and of the esoteric references, now confined to footnotes, none have been omitted that might be of use in higher degree theses. In the index, authors are more numerous than organisms, an indication of the widespread desire to emphasise the bibliographic aspects of biological research.

What will the student find? By the third page he will already be armed with a battery of words with which to confront his examiners. He will know that a living organism is a "protoplasmic system." Its transparent contents are always "hyaline," a network in a fixed nucleus is a "reticulum." He may later, if he reads Belar, learn to know it as an "artefact." But this is one of the words that has not got into the index. This is enough for a beginning; but words are very important in this study (Denn eben wo Begriffe fehlen da stellt zur rechten Zeit ein Wort sich ein). Later the *Introduction to Cytology* will tell the student what an atelomitic isobrachial heteropycnotic idiochromosome is. It will tell him how an undefinable chromonema winds its way through an illimitable matrix. Why there is no glossary we shall learn later.

Having learnt the passwords the adventurer will feel free to enter the realm of constructive study. If he is used to the reasoning of chemistry and physics that is his misfortune. For he must now be inducted into the less rigorous methods of an inexact science. Consistency here is less important and definition is always of the *ignotum per ignotius* type. The student must not be puzzled to find the modern trivalent of three chromosomes arising amidst the ruins of ancient tetrads of two chromosomes, for the Greek unit is half the Latin one and the roots are interchangeable. But if he is not familiar with the philosophic

system concerned he will be puzzled to learn that in this branch of biology all antecedent conditions are taken to be sufficient causes until the contrary is proved. Thus "synapsis is not solely a matter of gene homology but is influenced by some condition pervading the nucleus or cell generally." If the student is not too much taken aback by this he will proceed to learn something quite new about homology itself (in inverted commas): the pairing chromosomes "are 'homologous' not only because of their ultimate common origin but also in the sense that they perform similar rôles in the life of the organism" (p. 272). In the circumstances it is too much to ask for a glossary.

This book is therefore not merely an exposition of biological facts. It is a demonstration of a biological method now much in favour. But the method leads to trouble. It leads to a lack of rigour in observation and of relevance in arrangement. Now cytology is after all becoming a science, and observations must be constantly compared and their inconsistencies resolved. This particularly shows itself in the inductive-deductive aspects of the study concerned with mechanical and genetical theory. The theories of mechanics are here divided between chapters on chromosome morphology and crossing-over. The "kinetic bodies" observed with one fixation are said to lie in "insertion regions" found with another. They "take the lead" in anaphasic movement (p. 116) and one might almost suppose that the rest of the chromosomes follow willingly. Sharp's illustration of what are apparently meant to be these bodies shows them quadruple at metaphase (Fig. 63). But all facts are free and equal. On the same page Navashin's figure shows them double. The student may take his choice. Or he may prefer to follow the author's example and remain ambiguous on matters where authorities differ. Then "tetrads" at meiosis are said to "take up their metaphasic positions with their attachment regions in the equator" (p. 262). Strange to say, this never happens at meiosis. They lie as near to the equator as the tropics of Cancer and Capricorn. But it would be true of ordinary mitosis and the difference provides one of the critical distinctions between the two processes. No distinction of effect is made between primary and secondary pairing at meiosis, although the one determines segregation and the other does not even influence it. Small wonder then that the author finds it interesting "to speculate upon the possible relation between the synaptic 'attraction' and the later 'repulsion'" (note the inverted commas) and no wonder at all that he finds that "such speculations have as yet little of a definite nature to support them." Presumably this part of the book was written in 1931.

The illustrations of mitosis and meiosis are consistent with the author's timidity in dealing with the part of the subject in which he himself has specialised. There is one figure of genuine historical interest—a drawing of chromosomes by Hofmeister in 1848. But there are too many others which are neither very old nor very good. No one can complain at the author reproducing his own figures of mitosis (Fig. 73) but the writers from whose papers Figs. 153, 155*a* and 191 are taken might with kindness have been spared being confronted with the results of their earlier work.

It is in considering its relationship with genetics that the writer on cytology has his obvious opportunity of synthesis, and one is naturally interested, remembering his chapter in earlier editions on "Weismannism and Other Theories," to see what Sharp has made of it. There is a considerable account of data of genetics. Remarks on breeding experiments are found in chapters 16, 17, 19, 23 and 25, and short passages in praise of the chromosome theory and genetic methods encourage the reader and bring chapters 9, 18, 19, 23 and 26 to a close. But what the student needs is not just a statement of the principles of genetics but an application of them as a working method in cytological research and as a basis for the arrangement of its data. This he will seek in vain. No attempt is made to show how the chromosomes of an organism genetically control their own behaviour in every phase of life. No attempt is made to present the critical evidence for or against any theory of crossing-over. On the other

hand, conclusions which, if they were true, would have the utmost theoretical significance are casually stated without any consciousness of their gravity. For example non-homologous association "usually disappears before diakinesis" (p. 276). There is no evidence of its ever being retained at diakinesis. The consequence of this method is that no conclusions can be arrived at with regard to the genetic interpretation of chromosome behaviour in hybrids or polyploids, or in apomictic or other aberrant species—the word species is not even indexed. Equivocation is everywhere. On this foundation any attempt to use chromosome studies as a tool for genetical or taxonomic research is out of the question.

There are many who delight in the study of facts compiled with impartiality and uttered with authority. There are some who find that a logical nexus is a hindrance rather than a help to this study. To such this book can be recommended. For amongst them it cannot fail to be useful: it will save the teacher teaching and the student thinking.

C. D. DARLINGTON.

*Manometric Methods as applied to the measurement of cell respiration and other processes.* By MALCOLM DIXON, M.A., Ph.D., Sc.D., with a foreword by Sir F. G. HOPKINS, P.R.S. Cambridge University Press, 1934. $7\frac{1}{2}$ in. $\times$ 5 in. Pp. ix + 119. Price 5*s*.

Manometric methods of measuring gas exchanges were first developed on modern lines twenty years ago, and their utility in physiological research has been amply demonstrated. The reacting material, immersed in a small volume of fluid, is placed in a closed system, and either amounts or rates of gas exchanges may be accurately measured on a delicate manometer; similar vessels serve as controls. Various very ingenious modifications of the apparatus permit of the measurement of both oxygen uptake and carbon dioxide output on the same piece of tissue.

In this laboratory handbook Dr Dixon passes on the benefit of twelve years' experience in the design and use of the chief types of manometer available. He gives an account of the theory involved, and, what is perhaps of most use to the isolated worker, adds numerous details of technique which should minimise errors, and save much time. Those relating to the rate of shaking, and the limiting thickness of tissue are especially valuable. Care must be taken, however, in applying the formula of p. 50 in determining this limiting thickness $d'$ of the tissue used. The rate of respiration at the cut surface of some plant tissues is probably much greater than that of the internal cells; moreover quite high concentrations of oxygen may allow of fermentation. The formula does not take such facts into account, and probably only an empirical determination of the effect of tissue thickness on the R.Q. will be satisfactory in estimating $d'$. Manometric apparatus gives results quickly and is almost fatally easy to use, and it is all the more important that the theory and methods should be most carefully examined. Dr Dixon has carried out this task very thoroughly. It is his critical attitude, and the bringing together of the various scattered references which give the book its main value.

The use of manometry in plant physiology began with Warburg's well-known researches on photosynthesis and respiration in Chlorella. In the fifteen years since this was published, only a few workers (e.g. Genevois, Emerson, Arnold) have used his methods. The contrast with the rapid advance and numerous improvements in the methods described here in the field of animal physiology is most startling. It is typical of the unfortunate lack of correlation or even of sympathy between plant and animal physiologists, that no mention of a plant other than yeast or bacteria, occurs in Dr Dixon's book, although the utility of the methods along various other lines is pointed out.

Both the Warburg manometer and the differential Barcroft have been used in work on Photosynthesis. The difficulties are in supplying the plant with carbon dioxide, and in controlling the illumination and temperature. Warburg describes his own solution of these problems, but probably further research would lead to improvements.

Manometry may also be applied to the study of the respiration of the excised tissue of the higher plants, although few of them will withstand the extensive wounding or even maceration which is the recognised procedure in dealing with liver, muscle or brain tissue, and the effects of which are too often ignored by biochemists. It would seem that only by manometric methods will it be possible to bring our knowledge of plant respiration into line with the great and growing body of facts now collected for yeast and animal tissue. There is no doubt but that Dr Dixon's book will be of service in bringing about a wider and more intelligent use of the methods he describes.

J. S. TURNER.

*School Botany.* By MACGREGOR SKENE. 2nd edition. Oxford University Press, 1934. $7\frac{1}{4}$ in. $\times$ 5 in. Pp. vi + 278. Price 3*s.* 6*d.*

The early call for a new edition of this successful little book has enabled it to be extended and improved in detail, and one may sincerely hope with the author that its usefulness may thus be still further increased. It professes to cover the field of matriculation botany, introducing its readers to the structure and activities of plants mainly in terms of the angiosperm. In the new edition a chapter on other kinds of plants has been added and deals very briefly with conifers, ferns, mosses, algae, fungi and lichens.

Although the bulk of the book deals with its matter in a fairly orthodox manner, there is a certain freshness of treatment that makes it agreeable reading even to those who have read elementary botany books before. This will probably not surprise readers who are already familiar with Dr Skene's earlier publications. Most botanical handbooks tell you that photosynthesis was discovered by Joseph Priestley with the help of a mouse, a sprig of mint and a bell jar. Not many tell you that Priestley's thoughts were set to work in this direction by an entirely false clue given him by the landlord of an inn at Harwich. (Incidently not even Dr Skene tells you of the difficulty Priestley had in repeating his famous experiment.) In this special example it may perhaps be thought that the author goes rather far. Is it wise to point out to the learner at such an early stage the undeniable fact that many of the most fruitful discoveries have been arrived at more by lucky chance than by unimpeachable reasoning? At least it may be pleaded that this cruel "debunking" should not be extended to living workers.

Among much that is excellent and stimulating one major point of treatment seems curiously outmoded. Thus, on p. 105, "Plants often store up food for future use"; on p. 93, "Here is a new kind of leaf, very different in form from a foliage leaf, and doing quite different work"; there are also numerous chapter headings such as "The Flower and its Work." It may be argued, of course, that these phrases are literally correct, nevertheless we do not usually say that a mixed solution of salt and silver nitrate stores silver chloride; nor that iron and nickel do different work because their properties are not the same. The fact is that these words are liable to be misunderstood and never more than when describing plants to the human young. Rightly handled, a biological training may be the best specific against anthropomorphism, but there are obvious pitfalls.

The illustrations by Miss Ruth Weston are line drawings that for the most part can safely be shown to pupils as examples of what should be attempted, but we devoutly hope that none will adopt the method of flower drawing

applied to the buttercup and rose in Figs. 36 and 52. Surely it is time that this venerable error was interred. The opposite extreme in Figs. 38 and 39 (poplar and plantain) is not really much more informative, but a happy inconsistency has led to better results with the daffodil, primrose and some others.

A feature of this reissue that catches the eye is the abandonment by the publishers of their familiar blue and gold cover in which the first edition appeared and its substitution by a pleasant green ornamented with a leaf of *Aconitum napellus*.

W. O. JAMES.

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